A KINETIC ANALYSIS OF THE MECHANISM OF GLUCOSE STIMULATED INSULIN SECRETION

by

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A Kinetic Analysis of the Mechanism of Glucose Stimulated Insulin Secretion

SUMMARY

Storage and signal limited mathematical models for the insulin secretion process are described. The use of mathematical models in research is discussed. Kinetic *in vitro* perfused pancreas studies using glucose as a stimulator of insulin secretion are evaluated in terms of the models. The kinetics of secretion inhibition by diazoxide and diphenylhydantoin (DPH) are explained in terms of the models and the compartment model is modified to allow simulation of an "off response". Essential constraints for a satisfactory model are described. The above results are discussed as they relate to observed beta cell electrical activity, ion fluxes and metabolite requirements. The distribution and metabolism of insulin *in vivo* is studied experimentally and evaluated with models. These studies lead towards eventual synthesis of secretion models with ion flux models and whole body insulin kinetics models.

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I am indebted to Dr. G.M. Grodsky and Dr. H.D. Landahl both of whom gave me many hours of guidance, training and without whom this work would not have been done. I wish to thank Dr. R.I. Macey for helpful guidance and suggestions. The section of the thesis on Diazoxide and DPH was on work done with Dr. S.R. Levin, Dr. M.A. Charles and Dr. Grodsky. The work in the section on insulin distribution and clearance was done with Dr. J.H. Karam as well as Dr. Landahl and Dr. Grodsky. I also owe thanks to [redacted] M. Manning, M.A. Jones, L. West, R. Fanska, and [redacted] istance and to Dr. J.C. Spudich for advice [redacted] xis. I thank my wife, Edith, for her goo[redacted] for moral support. Thank you Dotty Fa[redacted] secretary.

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Abstract

Storage and signal limited mathematical models for the insulin secretion process are described. The use of mathematical models in research is discussed. Kinetic in vitro perfused pancreas studies using glucose as a stimulator of insulin secretion are evaluated in terms of the models. The kinetics of secretion inhibition by diazoxide and diphenylhydantoin (DPH) are explained in terms of the models and the compartment model is modified to allow simulation of an "off response". Essential constraints for a satisfactory model are described. The above results are discussed as they relate to observed beta cell electrical activity, ion fluxes and metabolite requirements. The distribution and metabolism of insulin in vivo is studied experimentally and evaluated with models. These studies lead towards eventual synthesis of secretion models with ion flux models and whole body insulin kinetics models. The following conclusions are made:

1. Present insulin secretion models must satisfy a complex series of events including fast, transient increased or decreased release, later phase, and hypersensitivity.
2. Specific signal limited secretion models work as well as specific storage limited models.
3. In addition to previously determined constraints (10) an additional hypersensitivity factor Q(G,t) (or equivalent elaboration) is required for sufficient insulin secretion models.
   (a) Q(G,t) has faster kinetics than the provisionary factor, P(G,t).

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(b) Release of catecholamines from nerve endings in the in vitro perfused pancreas does not account for the fast decay of hypersensitivity factor (Q(G,t)) at glucose=0.

4. The in vitro pancreas is sensitive to the rate of change of glucose concentration.

5. The action of diazoxide, including overshoot behavior when the drug is discontinued, can be simulated by a 3 compartment model; whereas a two-compartment model is sufficient to describe the action of DPH.

6. Linear compartment models of in vivo insulin distribution and clearance are not sufficient to simulate both high concentration bolus and steady infusion experiments with a single set of values for the constants

7. Models with a saturable pathway for the degradation of insulin are sufficient to simulate the clearance from the blood of insulin presented by high concentration bolus and by steady infusion using the single set of values for the constants.

8. No present insulin distribution and clearance model with a saturable pathway is uniquely defined by the present data.

9. Measurements of insulin levels following both a high concentration insulin bolus and a short steady infusion are necessary to adequately define the insulin metabolic characteristics of an individual patient.

10. A combination of a storage limited model and a signal limited insulin secretion model could simulate all of the experiments presented in this thesis, but such a model would not be unique.

R. D. Landahl
Chairman, Thesis Committee

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I. Introduction

The following is an analysis of the process of insulin secretion using secretion kinetics and other relevant data. Many different phenomena have been implicated as key processes in the mechanism of secretion. These phenomena are listed and an attempt is made to construct realistic models which could account for secretion in terms of such processes. The models are characterized as signal limited or storage limited. In so-doing it is hoped that these models will provide a structure upon which further research can be based.

This thesis does not provide the complete and final model for the process of glucose stimulated insulin secretion. However, one result of this study is a list of constraints for all sufficient models. The work here shows how models are used to define the limits of a problem, even though the data is not sufficient to determine the problem's unique and satisfactory solution.

Though the pancreas has an endocrine and an exocrine function, in this thesis, only the endocrine function is discussed. Several endocrine hormones are secreted from the islets of Langerhans found in the pancreas. They include somatostatin, glucagon and insulin. This thesis will directly discuss only the secretion of insulin although many of the conclusions relate to the secretion of other hormones. Approximately 1% of pancreatic tissue is in the form of islets. 80 or 90% of the cells in the islets are insulin secreting beta cells. Insulin is the only hormone which can directly lower blood sugar concentration; a defect in the secretion or action of insulin can lead to the disease diabetes mellitus.

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Insulin is a polypeptide hormone, which after synthesis is packaged by the beta cell into membrane bound vessicles somewhere in the Golgi region. Immediately after packaging the contents of the vessicles are not opaque but later as the contents crystalize dark granules appear under the light microscope. The bulk of the insulin in the beta cell seems to be stored in this membrane bounded granular form. Secretion is thought to occur via a process called exocytosis. In exocytosis the membrane of a vesicle fuses with the cell membrane and then the membrane breaks at the fusion point and the granule is released into the extracellular space.

In vitro pancreas perfusion studies of insulin secretion rate as a function of time during constant square wave infusion of glucose shows a multiphasic response (Fig. 3) with a fast early phase followed by a later slowly rising rate of insulin secretion. The late phase rises to a steady state in approximately 60 minutes (1-4). Similar multiphasic insulin secretion patterns during constant I.V. stimulation have been observed in intact animals and man (5-7). Studies show that mild diabetics may have impairment of only the first or both phases of insulin secretion (8,9).

At the time this work was begun there was only one detailed biologically descriptive "mathematical model" of pancreatic insulin secretion in existence (10), although there were several mathematical descriptions in the literature (11-13). That is, for a mathematical description to be a biological model it must have been conceptually associated with known or predicted biological processes to a reasonable extent. Early insulin secretion models were not often biologically very specific because they were usually a simplified, small part of an in vivo whole

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body glucose control model. However, some of the mathematical descriptions have been related more specifically to biological processes in later reports by their authors and thus their differences from biologically descriptive models have become less clear (14,15).

1. In a series of papers, G.M. Grodsky, H.D. Landahl and others have developed a model which could account rather well for the multiphasic insulin secretion response to an abrupt maintained step in glucose concentration (16-20,10). This model was a two-compartment model of the pancreas based on the assumption that insulin was stored in packets which varied in their secretion threshold to glucose concentration. One compartment* was labile and approximately 2% of a larger stable compartment. Other mathematical descriptions of the insulin secretion process include the following:

2. E. Cerasi produced a lumped variable analog computer description of the in vivo pancreas (11). In this case there was little attempt to correlate the biology to the mathematical functions and the effort could be described as rational curve fitting. This single pool model had a rate dependent early phase.

3. Porte and Pupo from in vivo studies suggested without a mathematical formulation a two-pool model for insulin storage and release (5).

4. Richard O. Foster presented a mathematical description of the in vivo pancreas assuming that there were rate dependent and concentration dependent phases of secretion (12).

5. Srinivasan et al. produced another mathematical description of the pancreas with a non-linear rate dependent early phase (13).

6. J.R. Guyton in a 1973 thesis produced an elaboration of the Foster and Grodsky models (above) (21).

*For this thesis no distinction is made between the terms compartment and pool.

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7. J. Tiran in his 1974 thesis produced a mathematical version of the Porte and Pupo model (above) (22).

8. E. Cerasi et al. produced a somewhat more biologically descriptive differential equation (negative feedback) elaboration of the analog computer model of Cerasi (above) (14). Other pancreas models or descriptions exist in the literature (23-27), but so far the most biologically descriptive models are the two pool models of Grodsky (7) and Tiran (22) both mentioned above.

The work in this thesis was based on the assumption that biologically descriptive secretion models are likely to be the most useful in the guidance of research into the biological mechanisms of secretion. Thus, the work that follows will be an attempt to explore biologically descriptive alternatives to compartment and pool models both to test the uniqueness and general applicability of these models and to establish the comparative validity of other biological explanations of insulin secretion.

In this thesis a two-compartment model has been modified so as to better correspond to more recent data developed with the in vitro perfused pancreas, and this modified form has been tested in various experimental situations. Experiments which were the most difficult for the two-compartment model to match were used as prime experiments in the construction of alternate types of models.

The in vivo distribution and metabolism of insulin in humans has been analyzed, modeled and tested experimentally in order to understand the biological significance of the observed secretion kinetics.

Ideally, the models shown here should not be thought of as a theoretical result, but rather as transient working models, or hypotheses, which each summarize our present understanding of a point of view of how glucose and stimulated insulin secretion may work. Of course it is quite

possible that certain aspects of these models will be retained far into the future. However, these models should be thought of primarily as an aide to the experimentalist.

II. METHODS

The *in vitro* experiments use the perfused pancreas. The technique for isolation and perfusion of the rat pancreas has been previously described (1,3). The pancreas, upper duodenum, stomach, and spleen were dissected from anesthetized, male, Long-Evans rats. This preparation was perfused through the celiac artery with Krebs-Ringer buffer and 4% human albumin (furnished by Cutter Laboratories, Berkeley, Calif.) with the aid of a constant temperature and oxygen control system. Perfusion rate was 10 ml/min. Glucose, with or without inhibitors, was added through a sidearm syringe. Glucose and drug levels were controlled by mixing weighed amounts of these substances into the sidearm syringes and infusing them at rates calculated to give the desired dose. Specimens for immunoreactive insulin assay were obtained from the portal venous effluent. The insulin assay is a standard radioimmuno solid phase assay modified from the method of Grodsky and Forsham (28).

6-OH-Dopamine treated rats were given 25 mg/Kg body weight 3 days before perfusion to destroy the adrenergic nerve endings in the pancreas as described elsewhere (29).

The computer work was done in Fortran on the U.C., S.F., IBM 360-50 and the Lawrence Berkeley Laboratory CDC-7600, and in Focal on the U.C., S.F. Department of Biochemistry and Biophysics PDP-12. Most simulation plots were done using a printer plotter routine written by Dave Karpinsky at the U.C., S.F. computer center.

The modeling process involved a cyclic interaction between experiment and computer simulations.

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III. Modeling the System

A. Purpose of Modeling

All scientific workers are involved in modeling. A hypothesis is a model. Thus, any reasonable experiment tests a model which exists in the mind of the experimenter. However, if the model becomes necessarily complex then the mental image may be less precise than the mathematical expression of the model. As a working model in a given research area becomes complicated, mathematical definition of the model together with simulation may be essential for comparison of the model to the data. In biomedical research the problem is often complicated by many factors which can be controlled but not eliminated. Thus, complex models are frequently required and mathematical modeling becomes a useful technique.

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III. Modeling the System

B. Techniques of Modeling

In each case it was attempted to associate the model as much as possible to the biological observations. First a model was conceived. Then the model was described mathematically. The following three different approaches were used to determine the values of the model parameters: (1) Pure trial and error simulation. In this case the modeler repeatedly adjusts model parameters to the best estimated value until a satisfactory simulation is achieved. (2) Least square fitting of isolated parts of the model to appropriate data. This method usually involves the assumption that certain aspects of the data are independent of other aspects. (3) Least squares fitting of all model parameters to the data at once. (a) Using analytic solutions, (b) using repeated simulations where analytic solutions, were not possible. This last technique (3b) requres the most computer time, however, for non-linear problems, 3a is often not possible.

In each case models are made no more complex than required by the data. Thus, if the data is definitive enough a unique solution for the values of the model parameters should be achieved. However, most of the model solutions presented in this thesis are not unique, and thus equally simple alternative models can be constructed and compared. Where the models are not unique constraints are listed which are common to all models tested.

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III. Modeling the System

C. Uniqueness of Biological Models

When any model is conceived, its uniqueness is a problem in common for every worker in every branch of science. In physics and chemistry the experiments can often be designed so that a unique model can be determined. However, the following factors make non-unique models common for the mathematical biologist. (1) The experimental apparatus is often provided by nature (in our case the rat pancreas). Thus,nature determines the minimum variability and complexity of the experiments. (2) Mathematical techniques are most often used for models which have become complex. Complex models are less likely to be unique.

When a model is not unique it is implied that a group of models are possible which could simulate equally well the available data. To eliminate the unsatisfactory models from the non-unique group more definitive experimental data must be obtained. The similarities and differences between the non-unique models can guide the experimenter in the choice of key experiments. Ideally after enough clever experimentation a unique model can be determined.

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III. Modeling the System

D. The Models Tested

1. General Approach

After much analysis it was decided that the two most useful opposing conceptual views of the pancreatic secretion system were that of a signal limited system and that of a storage limited system. For the most part a storage limited model existed at the beginning of this work. So that comparison of a modified version of the storage limited model to a group of signal limited models eventually evolved.

The following is a brief skematic description of each of the models. A detailed mathematical description appears in the appendix.

2. 2-Compartment Model

The 2-compartment model was developed by several workers in a series of papers, with one of the most recent versions published by G. Grodsky (7). In that version the model was shown in two alternative forms (Fig. 1a & b). Recent data on the synthesis-storage-secretion of insulin indicated that the b form was the more correct (30). The model had been tested in the a form, and thus the first step of this study was to write and test the b form, where synthesis is directly into the large compartment. It was found that the b form simulations did not differ significantly from the a form simulations. The b form is the version of the model which will be called the "storage limited" or "two-compartment model" throughout this thesis.

In the two-compartment model of the pancreas, insulin is stored in a labile compartment, containing 2% of the total insulin. The labile compartment is refilled from the larger stable compartment which con-

tains 98% of the total insulin. Insulin in both compartments is stored in packets which vary sigmoidally in their glucose threshold for release. The constant m controls the release rate of insulin from the labile compartment for packets whose threshold has been exceeded. Parameters $K_1(P)$ and K_2 control the rate of movement of insulin from the large to the small compartment and back respectively. Postulated provisionary parameter P is a function of glucose and time and controls the value of $K_1(P)$ and in doing so controls the rate of refilling of the labile compartment. Thus the model produces the early phase of multiphasic secretion by rapid depletion of the small compartment and the slowly rising late phase represents refilling from the large compartment controlled by the value of provisionary parameter, P. P is assumed to reach a steady state value in approximately 1 hour.

3. The Delta Signal Model

The delta signal model (Fig. 2a) is an attempt to associate early phase insulin release with the rate of change of a signal as opposed to release from a small compartment. The delta signal model is a one compartment model of the pancreas. The compartment is assumed not to be significantly depleted during a one hour experiment. The compartment volume has been lumped into the constants and thus the insulin secretion kinetics are represented by the kinetics of the parameter controlling release from the compartment. In this case it is assumed that a temporary gradient is immediately set up across a structure in the beta cell when the cell is presented with a rapid upward step in glucose concentration. The temporary gradient is assumed to be the signal for early phase insulin release. This model includes a postulated provisionary factor, P,

identical to the factor assumed in the two-compartment model as the primary controller of late phase secretion. However in this model the factor is used to modify the release constant.

4. Exciter-Inhibiter (Feedback) Model

The exciter-inhibiter model (Fig. 2b) is based on the following assumptions. A fast upward step of glucose concentration triggers a rapid rise of the concentration of an exciter in the cell. The exciter (or the glucose) triggers a rise of an inhibiter which rises to a concentration approaching that of the exciter. The inhibiter inactivates the exciter resulting in the transient early phase release; the rising late phase of secretion is controlled by a postulated provisionary factor, P, identical to the factor in the 2-compartment and the delta signal model.

Mathematically the exciter-inhibiter (feedback) model and the delta signal model differ only slightly. Both are one compartment signal limited models which operate thru control of a release parameter. The difference between the exciter and inhibiter is analogous to the size of the gradient in the delta signal model. However, the gradient in the delta signal model occurs immediately when stimulated by glucose, whereas the exciter requires a short but finite time to build up. This mathematical difference has no significant effect on simulations in this thesis. Thus the major difference between the delta signal model and the exciter-inhibiter (feedback) model is conceptual.

Since the predictions are equivalent all signal limited models simulations in this thesis are done using the delta signal model, but are referred to as "delta-feedback" model simulations.

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IV. Results Section

A. Kinetic Glucose Studies

1. Introduction

The insulin secretion response of the pancreas to a constant step of glucose concentration is multiphasic (1-4) and is therefore a complex response. In this study it is assumed that by careful examination of these and other complex secretion patterns one can gain insight into the fundamental mechanisms of insulin secretion. The method used is to compare different quantitative models derived from certain kinetic control patterns of insulin release and subsequently test them with a variety of other stimulatory conditions.

The basic control pattern consists of constant steps of varying glucose concentrations continuously perfused for 1 hour (Fig. 3). All rate constants, compartment sizes and other parameters are fixed from these basic experiments. The test patterns include staircase infusions (Fig. 4), pulse experiments (Figs. 5,7,&8), ramps (Figs. 12-13) and others. The models used will be the two-compartment and the delta-feedback models described in the modeling section above. These models are thought to be conceptual opposites, i.e. a storage limited model versus a signal limited model. It should be stressed that the storage limit mentioned above does not mean a limit in the rate of synthesis (which is known to be slow) but rather a limit in the movement of insulin stored in the cell to a site available for secretion.

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2. Results

All models were fitted to and produced satisfactory simulations of experiments where different glucose concentrations, G, were held constant for 60 minutes and insulin secretion measured (Fig. 3). That is, in the two-compartment model, the size of the small compartment and its distribution of threshold sensitive packets were chosen to produce the first phase response observed in Figure 3. Parameter values controlling the response of the delta-feedback model to a change in glucose concentration were also chosen to match the experimental first phase behavior shown in Figure 3. In both models the second phase behavior was controlled by the identical postulated provisionary factor, P, whose value at 60 minutes was matched to the observed secretion rates after 60 minutes shown in Figure 3, which were assumed to be steady state values. Thusly, P was defined as a slowly responding factor. Without further adjustment of any constants in either model, all staircase simulations were within experimental error (Fig. 4). For all models the staircase simulations were similar because refilling, which is the major difference between the two-compartment model and the delta-feedback model, is not the dominant factor in that experiment. In ascending staircase experiments, according to the analysis used in the models here, one observes secretion from previously unstimulated packets or cells, or because higher glucose levels stimulate higher signal levels.

In the experiments where glucose was infused for 5 minutes at G=100mg% and then stopped (G=0) for 5 minutes alternately, only the delta-feedback models gave a satisfactory simulation (Fig. 5). In this case the two-compartment model with the rate constants developed from data in Fig. 3 could not be made to refill the labile compartment rapidly enough to account

for the large spikes of insulin secretion seen. The delta-feedback model simulates this experiment successfully because the capacity to secrete a normal first phase of insulin secretion is recovered quickly enough in that model. In the delta-feedback model recovery of first phase spiking ability is not bound to the value of the postulated provisionary factor, P, via refilling as it is in the two-compartment model. That is, in the two-compartment model, the rate of refilling of the small compartment is controlled by the balance between rate constant from the large to the small compartment (a function of P) and the rate of release of insulin from the small compartment, whereas in the delta-feedback model the rate of recovery of spiking ability is dependent only upon the time since the last change in glucose concentration. Effectively the delta-feedback model increases the rate of total provision during repeated fast increases and rests of glucose concentration beyond the value predicted by the two-compartment model.

To test the negative spike producing capacity of the model an experiment was performed and simulations attempted using G=500mg% for 20 minutes, G=150mg% for 20 minutes, G=500mg% for 10 minutes, and G=150mg% for 10 minutes (Fig. 6). The experiment shown in Figure 6 was designed to produce maximum "refractory" effect or "negative spike undershoot" in insulin secretion rate obtainable by abruptly reducing the glucose concentration to a lower though still stimulating concentration. For this experiment (and simulations of all other experiments) it was not possible to produce a negative spike with the two-compartment model. The reason for this is that the release constant of the two-compartment model does not change when the glucose concentration is lowered. Initially, the insulin secretion rate decreases as the glucose concentration falls below

the glucose threshold for some of the cells (or insulin packets). The second effect is the slow movement of the provisionary factor, P, to its steady state value for the new glucose concentration. The behavior of the release constant may not be changed in the two-compartment model, because a constant release rate is necessary to fit the control curves where the early phase duration is a constant independent of glucose concentration. The rate of change of P in response to a change in glucose level could not be altered in the model because it was fundamentally linked to the slow second phase insulin secretion behavior shown in Figure 3. For this experiment, the early phase part of the delta-feedback model release constant becomes temporarily negative and gives a distinct undershoot at 20 and 50 minutes. This undershoot occurs because the direction of the gradient is reversed in the delta signal model, and the inhibiter concentration is temporarily greater than the exciter in the exciter-inhibiter (feedback) model.

The experiment also showed a behavior called hypersensitization. That is the spike seen at 40 minutes in Figure 6 is larger than could be accounted for alone by provisionary factor, P. The delta-feedback model can not simulate hypersensitization reflected by the spike at 40 minutes, because the gradient produced by the glucose change at 40 minutes is less, not greater, than at zero time, because the size of the glucose change is less and because increased P only provides an increased baseline and has no direct effect on spike amplitude. The two-compartment model does produce such a large spike due to its property of hyperfilling of the labile compartment with high threshold packets during second phase secretion. Thus, a modification was made in the delta-feedback model to introduce into it second phase hypersensitivity.

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The modification of the delta-feedback model involved the addition of an insulin secretion term which was a function of the product of the provisionary factor and the effective gradient caused by a change in glucose concentration. The modified delta-feedback model was then fitted to the experiments shown in Figures 3 and 6.

In further testing both the two-compartment model and the simple delta-feedback model produced a satisfactory simulation of the experiment where G=300mg% for 60 minutes, G=0mg% for 5 minutes, and then G=300mg% again for 5 minutes (Fig. 7). However, the modified delta-feedback model produced a spike at 66 minutes which was approximately a factor of three too large. This experiment showed that modification of the simple delta-feedback model hypersensitized the model's second phase behavior too strongly. This result implied an additional complexity in the behavior of the pancreas which will be discussed below.

The models were then tested against an experiment where glucose was perfused for short periods at G=300mg% and then for short periods at G=0mg% alternately (31) (Fig. 8). For this experiment the delta-feedback model gave the most satisfactory simulation. The two-compartment model was not able to refill the labile compartment swiftly enough (without a modification of the small compartment size) and the modified delta-feedback model showed a too rapidly increasing second phase hypersensitivity.

With the above results in mind three new sets of experiments were designed. As a control for the effects of glucose concentration in the experiment shown in Figure 6 a similar experiment was done with the upper G value reduced to G=300mg% (from G=500mg%). The result of this experiment was much the same as the experiment shown in Figure 6, i.e. only the modified delta-feedback model fit the data in a satisfactory

mannor (Fig. 9). Next the experiment shown in Figure 6 , in effect, was done replacing the first 20 minute infusion of G=500mg% with G=150mg%. The result was a markedly reduced spike at 40 minutes when G was changed from 150mg% to 500mg% (Fig. 10). However, after a 10 minute wait at G=150mg% another G=500mg% squarewave produced a spike twice as large as the previous spike. This led to the hypothesis that "hypersensitivity" or "potentiation" of the early phase type of insulin secretion to fast changes in glucose concentration may be a process in addition to and/or independent of the value of provisionary factor, P.

The experiment shown in Figure 10 suggests that 10 minutes of exposure to 500mg% glucose followed by 10 minutes at 150mg% glucose is enough to stimulate hypersensitivity, even though the value of P has not changed enough to account for the difference in either the two-compartment model or the delta-feedback model. Hypersensitivity in the two-compartment model depends on the value of the rate parameter controlling the rate of flow of insulin from the large to the small compartment which is controlled by the value of P. Hypersensitivity does not really exist in the delta-feedback model although the P provides an increased baseline which makes spikes of insulin appear larger when P is large. A variable hypersensitivity factor, Q(G,t) which responds faster than and is independent of P is a possible explanation of increased sensitivity of the pancreas to squarewaves of high glucose concentrations (Fig. 6,9,10, and 11) - a sensitivity increase which is dependent upon the pancreas having been exposed recently to a high glucose concentration. That is, the behavior observed in Figures 6,9,10 and 11 could be explained by a stimulation of a hypersensitivity factor which quickly becomes available after exposure and remains available for at least twenty minutes. It was notable that the experiment shown

in Fig. 10 was matched more poorly by the two-compartment model simulation than were the experiments shown in Figures 8 and 9. In fact in Fig. 10 the relationship of the two G=500mg% spikes were reversed in the two-compartment simulation, i.e. in the simulation the first spike was far larger than the 2nd spike. Thus, the simple provision and potentiation observed in the two-compartment model is not sufficient to account for all observed sensitivity increases of the pancreas. For the experiment shown in Figure 10 the refilling time is not long enough and the value of P not large enough for the model to produce that large a final spike of insulin secretion. For the experiments shown in Figure 10 both the delta-feedback model and its modified version did much better than the two-compartment model but neither of them gave an entirely satisfactory match.

Finally, a third time control experiment was done which lent further support to the hypothesis that an additional hypersensitivity factor exists which may be independent of P and which responds to high glucose concentrations more quickly than P. The time of the initial G=150mg% period in experiment 10 was reduced from 40 to 20 minutes, yet the size of the two G=500mg% spikes showed little change in absolute or especially relative magnitude (Fig. 11). The simulations matched roughly as before for the experiment shown in Figure 10. This supported the conclusion that the 2nd phase results observed were not so much time dependent as dependent upon the type of glucose fluctuations to which the pancreas was subjected.

Ramp experiments were simulated (Fig. 12 & 13). In the slow and fast ramp experiment simulations shown in Figures 12 and 13 respectively it could be seen that such ramp experiments did not distinguish well between the two-compartment model and the delta-feedback models, because all

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models gave similar satisfactory simulations for such experiments. In the experiment shown in Figure 14 the glucose concentration was increased smoothly from G=40mg% to G=100mg% over the first 29 minute period, then G was held at 100mg% for 10 more minutes; G was reduced to 0mg% for 5 minutes and finally G was returned to 100mg% for 5 more minutes. The two-compartment model simulation showed essentially only low secretion at any time during the experiment. Whereas, the delta-feedback model, which showed low level activity during the ramp to plateau part of the experiment, and then showed a distinct fast spike of insulin secretion in response to the final squarewave of glucose. The same type of fast spike is observed experimentally for all models when presented without a preceeding ramp (Fig. 3). The two-compartment model could not produce the type of fast late spike shown in Figure 14 because its labile compartment was depleted by the early ramp to plateau of glucose concentration portion of the experiment and this labile compartment could not refill rapidly enough at the low provisionary levels that exist within the two-compartment model for G=100mg%. It should be emphasized that the ramp portion of this experiment did not distinguish between models. Differences seen in simulations during the restimulation were similar to those of the other restimulation experiments (Fig. 5 and 8).

3. Evaluation of the Hypersensitivity Factor

In an attempt to understand the hypersensitivity factor postulated above it was noted that Iverson et al. (32) had seen an increase in epinephrine release from the in vitro perfused dog pancreas in the absence of glucose. The epinephrine release was inhibited by higher glucose. It is known that epinephrine can inhibit insulin secretion and so we hypothesize that the epinephrine produced at low glucose concentrations was the reason the hypersensitivity factor did dissipate when the glucose

concentration went to zero for 20 minutes. A catecholamine assay was not available so the Iverson values for the dog were assumed to be correct for the rat. Thus, the experiments shown in Figures 15,16, and 17 were performed. Figure 15 is the control experiment somewhat similar to the experiment shown in Figure 6 except that it was done with the purified perfused pancreas preparation described in section IV.B.2. below. Figure 16 shows an experiment which differs from the one shown in Figure 15 in that glucose concentration drops to 30mg% between 20 and 40 minutes instead of 0mg% as in the control. Figure 17 shows an experiment like the control experiment except that the rats were pretreated with 6-OH-Dopamine to destroy adrenergic nerve endings as described in the methods section. Analysis of the experiments in Figures 15,16, and 17 showed no significant differences, suggesting the reported epinephrine release from adrenergic nerve endings at low glucose concentrations is not the explanation for the dissipation of hypersensitivity phenomena seen above.

4. Discussion

Both the two-compartment model and the delta-feedback model (and the modified delta-feedback model) matched most of the data, but all of the models had difficulty (not always the same difficulty) with certain experiments, however, each model was helpful in the analysis of the experimental results. Table 1 shows a summary of the performance of the models for each type of experiment. In general it appeared that the gradient type of model (delta-feedback model) provided as satisfactory a description as the two-compartment model of the behavior of the pancreas. The original delta-feedback model has no potentiation in it. A simple modification of the delta-feedback model's second phase behavior described above is not sufficient to allow complete description of the

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second phase (potentiation) behavior of the pancreas in all cases. To match all of the experimental data it will be necessary to modify the second phase behavior of the delta-feedback model in some way so that it has the second phase hypersensitivity observed in the in vitro pancreas.

The above results indicate that, to simulate the hypersensitivity correctly, an additional factor must be included which may have kinetics independent of the slow kinetics of provisionary factor, P. The factor must build up within 10 minutes at 500mg% glucose, it must stay up for 20 minutes, and it must dissipate rapidly when the glucose concentration reaches zero or near zero. An alternate explanation might be an increase in first phase sensitivity to P under the above conditions. However, either change would be an equal complication to the altered model. Characterization of this behavior is a more complex experimental problem than those modeled above and much more experimental data would be required to describe it quantitatively in a model.

The results of simulations by the delta-feedback model including negative undershoot in insulin secretion (Fig. 6,9,10, and 11) and in simulation of spike secretion of insulin for low pulses of glucose concentration (Fig. 5 and 14) strongly suggest a sensitivity to rate of change of glucose concentration in the mechanism of insulin secretion. This will be discussed in detail in a later section. This aspect of insulin secretion is consistent with electronic studies of pancreatic beta cells (33,34) which show that depolarization of beta cells and the number of beta cells initiating action potential discharges increase with increasing glucose concentration. The ion requirements of beta cell electrical excitation are similar to many requirements observed in the perfused giant squid axon (35,36). Thus, by analogy if these beta cell

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action potentials are required for first phase spike insulin secretion, the disappearance of fast spikes of insulin secretion when the glucose concentration is increased slowly may be due to electrical accommodation of the beta cells in the population to the depolarization by glucose. Conversely the negative undershoot observed when the glucose concentration is dropped quickly to a less effective glucose concentration may be due to hyperpolarization of beta cells in the population (37).

The delta-feedback model is a rate dependence model. It is consistent with secretion control being accomplished by a rapid flow of ions of Ca^{++}, Na^{+}, and K^{+} and the corresponding temporary electrical change. The delta-feedback model is mathematically similar to the two-factor model of Rashevsky and Landahl which was found to have many of the properties of electrically excitable cells (38,39). However, there is little doubt that a sufficiently complex version of the two-compartment model could also be matched to the data. (e.g. Guyton in a thesis produced negative spikes with a complex 4 compartment alternate to the single labile compartment in the two-compartment model used here.)

Ramp experiments were found to be no test of the two models. This is because the threshold distribution hypothesis is essentially equivalent to simple derivative rate dependence, at least for the first stimulation before refilling comes into play.

Feedback inhibition is still a possible model for the insulin secretion process. The exciter-inhibitor concept shows that this type of model can simulate the data as well as the other models. The actual biological processes represented by the exciter and the inhibitor are yet to be determined with certainty. Several candidates exist and will be discussed in the general discussion section at the end of the results section.

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All models have the same kind of second phase controlled by the same postulated provisionary factor, a mechanism stimulated by glucose but different from that responsible for first phase release. This is consistent with experimental observations where the first and second phases can be differentially stimulated or inhibited by various chemical agents (1,40,41,42).

IV. Results Section

B. Rate Dependence

1. Introduction

As noted glucose, when presented to the pancreas as a rapid continuous single step, causes a fast but transient release of insulin that terminates during the first 5 minutes of the maintained stimulation (Figs. 1-7). Subsequently, a second phase occurs, characterized by a slowly increasing rate of secretion, Similar multiphasic responses are also seen in other endocrine systems (43,44,45). Some mild diabetics have impairment of only the early phase of insulin secretion (8,9), and Albisser et al. have shown that a fast release is necessary for minimizing the insulin required for accurate blood glucose control by a mechanical pancreas (46).

A sigmoidal dose-response curve is observed when total insulin released in either phase is plotted against glucose concentrations used in a series of single-step stimulations (10). These types of studies, however, do not evaluate the importance of the rate of change of the stimulator as a signal. When glucose is presented as a linearly increasing ramp, the first phase appears to be smaller but is more prolonged with the decreasing slope of the ramp and ultimately becomes difficult to measure in the presence of the gradually increasing second phase (Fig. 10). Cerasi (11), Foster (12), and Srinivasan et al. (13) incorporated a rate-of-change signal for first-phase insulin release in a pancreatic whole body model, but this was not required in equally effective computer models developed from in vitro pancreatic studies (10,17). Although studies involving comparisons of ramp and step glucose concentration changes on first-phase insulin secretion show a clear change of

pattern, they have not established whether a fast change in glucose concentration causes more total insulin secretion than a slow change. (This is equivalent to asking what has happened to the early phase in the slow ramp, where it is no longer discernible.) Experimental design is complicated by the following three special problems: 1) insulin release is a non-linear function of glucose concentration; 2) the insulin response to glucose produces an overlapping second phase; and 3) the full response of insulin to a given change in glucose concentration requires 4 to 5 minutes (10). Previous studies relating to the above question could not easily control these problems because they were performed in vivo (47) or were designed for other purposes (48,49).

2. Methods

The in vitro perfused rat pancreas preparation with non-recirculating phosphate-bicarbonate buffer containing human serum albumin (1%) and dextran (4%) were used, as previously described in detail (3). The flow rate was 4 ml/min. The experimental design for comparison of glucose presentation as a ramp or step configuration is shown in the upper portions of panels a and b of Fig. 18.

The limits of the change in glucose concentration (from 110 to 180 mg/dl) were chosen to restrict studies to the approximately linear region of the sigmoidal dose-response curve previously established with this preparation (10).

Additionally, 110 mg/dl is a good base level because at that glucose concentration there is little late-phase insulin secretion to complicate measurement of first-phase release (10) (see lower portions of 18a and 18b).

As previously demonstrated (10,17) and shown here (lower portions of 18a and 18b), about 5 minutes are required for full insulin secretion at

a maintained glucose step. After that, particularly at high glucose concentrations, late-phase secretion will interfere. Thus, in comparing both cases, insulin secreted for 5 minutes after the end of the change was included.

It was to be expected that comparing ramp and step periods longer than 5 minutes from the end of each change would show a steadily decreasing percent difference in total secretion and that using less than 5 minutes would be to the advantage of the ramp because the second step of the two-step change would not have had time to give its full early phase response. Examination of the data confirmed this. All constraints favored either seeing no effect or seeing greater secretion from the ramp, and thus any rate dependence indicated by increased release by the two-step stimulation may not be the full value.

3. Results

During the experimental period, glucose was increased from the base level of 110 mg/dl to 180 mg/dl in two steps (35-mg/dl increments of 5 minutes duration) or as a ramp (eleven 6.36-mg/dl steps of 1/2 minute duration). Thus, in comparing the two-step change with the ramp change, the limits are in the linear portion of the curve and are the same. Also, the average concentration of glucose during the respective experimental period period, the total amount of glucose going through the pancreas, the total time of the change, and the average rate of change are the same. The only difference is the path (i.e. the size of the step changes or the maximum rate of change, which is 5.5 times greater in the two-step than in the ramp stimulation); the time required to make a concentration change was essentially the same for each of the two large glucose steps as it was for the eleven small glucose steps. To control the possible

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influence of sequencing, the two-step stimulation either preceded (panel 18a) or succeeded (18b) the ramp. A reproducible spike of insulin release occurred with each of the two-step stimulations; the ramp experiments gave no significant spikes but showed a progressive rise of insulin of more variable magnitude among experiments.

As shown in Fig. 19, in the five experiments in which the two-step change was first, it stimulated secretion of 50% more insulin than the ramp. In the five experiments in which the ramp change was first, the two-step change still produced 25% more insulin secretion. When the results of all experiments were compared, it was found that 36% more insulin was secreted in response to the two-step change than to the eleven-step ramp. The data suggest a maximum-rate dependence for glucose-stimulated insulin release which, under the conditions of these experiments, can account for more than 30% of the glucose-related first-phase insulin release.

4. Discussion

In these experiments it is not possible to observe directly the time of onset of late-phase secretion. However, 5 minutes after the end of the change the average secretion rate is actually 23% (not significant) higher for the ramp than for the two-step change. This possible difference suggests that a larger amount of late-phase secretion is not the reason why fast changes stimulate more release.

We know of no other endocrine system that has been studied in this way. However, rate sensitivity has been discussed in general by several authors (50-53) and specifically with regard to bacterial chemotaxis (54), liver adipose cells (55), the pituitary adrenal system (56-58), and the pancreas (59). Other biological systems may act similarly: e.g., a slowly rising current, much greater than a rapid increase, can depolarize

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a nerve axon without triggering an action potential (60). This type of rate dependence requires at least two processes which proceed at different rates. Among the several types of models that produce this type of behavior qualitatively are the following: 1) a model with a labile compartment that begins to "leak" some of its contents slowly back to a stable compartment when secretion is stimulated (and, thus, slow glucose concentration changes would cause a greater loss of available insulin from the labile compartment than fast changes); 2) a Hodgkin-Huxley type of model, which has a fast and slow gate (61); and 3) a model in which a rapidly rising exciter and a more slowly rising inhibitor of secretion increase to their respective steady states in response to glucose stimulation, but with a threshold in the exciter-inhibitor difference required for secretion or with secretion dependent on the amount of exciter-inhibiter difference raised to a power greater than one.

The rate-sensor property demonstrated for the pancreas is advantageous for effective control systems, and thus may prove characteristic of various biologic homeostatic mechanisms.

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IV. Results Section

C. Diazoxide and DPH

1. Introduction

Diphenylhydantoin (DPH), a drug that reduces the frequency of seizures, can also inhibit insulin secretion in man (62-65) and in laboratory pancreatic preparations (66-68). Another drug which inhibits insulin secretion, diazoxide, has been used purposely to elevate the blood sugar in certain hypoglycemic states (69-72). At least part of this effect of diazoxide is related to inhibition of insulin secretion, an effect that has also been demonstrated in vitro (73-75, 68, 76).

In other studies the responses to DPH and diazoxide were compared during the second phase of glucose-induced insulin secretion (68). It was shown that concentrations of either agent, carefully selected to cause a prompt initial inhibition of the second phase of the same magnitude, produced characteristically different responses when its perfusion was maintained during 20 minutes of a continuous, 60-min, 300 mg/100 ml glucose perfusion. DPH caused constant inhibition with return to preperfusion secretion levels immediately after discontinuation of the drug. Diazoxide inhibition was characterized by a transient inhibition followed by a gradual escape; termination of the drug resulted in a large overshoot of insulin release. In the compartmental model, assuming a dual action of glucose both on release and on provision of additional insulin (10,17), the primary action of DPH was consistent with a generalized inhibition of all phases of glucose action, both on release and provision. In contrast, the effect of diazoxide appeared to be at, or close to, the final release mechanism, with little effect on provision. In a series of experiments we

have expanded previous studies to contrast further the effects of these inhibitors, particularly on the initial phase of insulin secretion. In addition, we have used the observed stimulus-inhibitor interactions to formulate possible mechanisms of inhibitor effect and to test, with the computer, the two-compartment hypothesis for insulin secretion.

2. Results

Experiment 1. Qualitative effects of inhibitors on early and late secretory phases of glucose-induced insulin secretion are shown in Fig. 20. Previous studies showed that diazoxide (75µg/ml) and DPH (25µg/ml), infused during the second phase of a glucose stimulation (300 mg/100 ml), produced almost complete and similar initial inhibition. Thus, these drug doses were used in the present experiments.

As shown in Fig. 20, glucose alone caused biphasic insulin release. The simultaneous addition of one of the inhibitors with glucose caused their respective characteristic (68) inhibition of the second phase: for DPH, second-phase inhibition (about 95%) was maintained and secretion returned to normal after termination of the infusion; for diazoxide, escape from inhibition occurred within a few minutes and, after termination of perfusion, there was a large overshoot (i.e., a transient hypersecretion). First-phase release persisted, though it was reduced. The following experiments investigated more closely the effects on the early phase.

Experiment 2. Effects of preaddition of inhibitors on early release are given in Fig. 21 and Table 2. In a series of separate experiments, the effect of preexposure to DPH and diazoxide were compared. The influence of each drug on the response to glucose was studied at two different doses (25 and 75µg/ml). Drugs were started simultaneously with or preinfused for 10 minutes before a 5-min infusion of glucose. Results were analyzed by

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summating insulin values obtained during the first 2-5 min, the summated insulin (Σ 2-5 immunoreactive insulin (IRI)).

Control glucose infusions caused typical, early "spike" discharge (Fig. 21a). DPH, 25µg/ml, when started simultaneously with glucose (Fig. 21b), inhibited summated insulin. Preinfusion of the same dose of DPH for 10 minutes further inhibited summated early insulin, compared to infusions in which DPH and glucose were begun simultaneously ($P<.001$). Raising DPH to 75µg/ml caused no greater inhibition than that achieved by DPH preinfused at 25µg/ml.

Diazoxide, at 25µg/ml and 75µg/ml, inhibited summated early insulin secretion (Fig. 21c). The preinfusion of diazoxide at these doses also inhibited summated first-phast insulin (Σ 2-5 IRI). However, the inhibition of Σ 2-5 IRI was no more than with simultaneous infusion of diazoxide, 25µg/ml or 75µg/ml respectively.

Thus, inhibition by low-dose DPH was time and dose dependent, i.e., preinfusion produced further inhibition. With diazoxide, however, effects were dose dependent, that is, preinfusion of the low dose did not increase inhibition. Table 2 statistically summarizes the results of experiment 2.

Experiment 3. Influence of inhibitors on response to sequential glucose pulses. In this experiment (Fig. 22 and Table 3) glucose stimulations (pulses I and II) were for 7 minutes with two 10-min rest periods, without glucose, after each stimulation. Inhibitors were continuously perfused 10 min before and during the two sequential stimulations. After the second rest period, the inhibitor was stopped and glucose alone was given for 10 minutes (pulse III).

In control experiments (Fig. 22a), early release was similar in response to all three pulses. When either inhibitor was used, there was inhibition

of summated release during glucose pulse I compared to control values. However, the effects of DPH (Fig. 22b) differed quantitatively from those of diazoxide (Fig. 22c). The response to pulse II was less than in pulse I with DPH. On the other hand, with diazoxide present, after inhibition of the insulin response to glucose pulse I, no further inhibition in summated response occurred during the second pulse. With pulse III (inhibitor removed) both inhibitory systems showed recovery, i.e., more insulin was secreted compared to pulse II. However, summated early secretion in response to glucose pulse III was greater in control experiments than when DPH ($P<.001$) or diazoxide ($P<.04$) were present, indicating that recovery from inhibition was incomplete. In addition, the response to pulse III in the DPH-treated pancreas was less than in the diazoxide-treated preparations ($P<.01$). The results of experiment 3 are summarized statistically in Table 3.

The studies expand on previous observations that DPH and diazoxide have qualitatively different mechanisms of inhibition (68). These differences were evaluated by the two-compartment model previously described and illustrated in Fig. 1b.

As shown in Fig. 23a, the present hypothesis for the effect of diphenylhydantoin involves reduction by the drug of several components of the system. These include terminal secretion (m), the effect of provision (δ), and a reversal of the flow of insulin (increased K') so that the trend is for insulin to move toward or remain in the stable compartment.[1]

Fig. 24, a-c, demonstrates the computerized version of the control experiments and compares the control simulations with theoretical plots of all experiments with inhibitors. The size of the labile compartment has been reduced to match the control data early phase. These plots closely approximate the experimental observations with DPH: first-phase secretion

was partially inhibited (decreased m); preexposure to drug enhanced inhibition (decreased availability of insulin in the labile compartment due to the time-dependent effects of continued decreased γ and increased K'); and there was no overshoot after discontinuation (though provision was stimulated normally, it was ineffective until discontinuation (normal P, decreased γ)).

As seen in Fig. 23b, our present hypothesis of the effect of diazoxide involves separation of the labile compartment into two compartments. The smallest subdivision contains 0.002% of total insulin, and the larger, 1.988%. Both have the same qualitative packet threshold distribution. Division of the labile compartment was necessitated by an apparent paradoxical difference in the effects of diazoxide, depending on whether it was added before or during the second phase of secretion. Diazoxide, added during the second phase, caused transient inhibition escape and overshoot after termination (indicating a buildup of insulin in the labile compartment by inhibition of terminal release (decreased m)) (68). However, in these present experiments low drug concentration (25µg/ml) only mildly inhibited the release of labile insulin during the first phase of glucose stimulation (m comparatively unaffected). Only when diazoxide was increased to 75µg/ml was reduction of m apparent, inhibiting total early insulin (Fig. 21). Response to a third glucose pulse after removal of diazoxide (Fig. 22, Table 3) showed recovery in summated

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[1]Reversal of flow of insulin should not be taken literally, since destruction ($\propto$) of insulin from the labile compartment is a mathematically equivalent phenomenon and could be a possible effect of DPH. The major suggestion is that insulin in the labile compartment is reduced in a fashion which is not associated with accumulation of insulin during the inhibitory phase in contrast to diazoxide.

early release. This again suggests involvement of sites closer to the provisionary phase, with sparing of more distal mechanisms (m relatively unaffected). Modification of the theoretical model to include the action of diazoxide closely approximated all experimental data (Fig. 24, a-c), assuming this drug inhibits transfer of insulin from a normally insignificant intermediate compartment (decreased K_+) with other processes remaining comparatively unchanged. Thus: first-phase secretion was comparatively unchanged (normal to slightly reduced m); second-phase release was transiently inhibited (decreased K_+); escape occurred (normal provision to the labile compartment with normal γ (effect of P) and K); preexposure to drug had no effect (no change in availability of insulin in labile compartment, normal γ and K); overshoot appeared after discontinuation of the drug (release of previously decreased K_+, with normal provision (P), K, and γ).

3. Discussion

Based on the two-compartment hypothesis, computer analysis has previously approximated a variety of experimental designs related to glucose-induced stimuli (10). In the present experiment, we have superimposed the inhibitory effects of DPH and diazoxide on the stimulatory effects of glucose. Each inhibitor had differential effects on the two phases of insulin secretion. As seen in experiment 1 and in previous experiments (68), incomplete inhibition of the first spike occurred at concentrations of DPH which completely inhibited detectable second-phase insulin secretion. With diazoxide, even at 75μg/ml, there was distinct early secretion, though this concentration initially inhibited second phase insulin secretion to almost undetectable levels.

These data illustrate that the two phases of insulin secretion reflect completely different phenomena, a conclusion consistent with that previously

suggested on the basis of differential stimulation of the two phases by glucose and tolbutamide (1) and their differential sensitivity to puromycin (3).

Using data derived from observations of the effects of the inhibitors during the late insulin release phase, it was suggested (68) that DPH could act by retarding all positive elements with the system, resulting in constant inhibition and no overshoot after drug removal. It was also suggested that diazoxide inhibited either release from the small compartment or a late phase in provision while much of provision continued. Thus, initial inhibition behind the blockade produced by the drug would be followed by gradual escape as provision increased. After drug removal, an overshoot would be observed. Current experiments showing that the first phase is only partially reduced by these drugs, at levels by which the second phase is almost completely inhibited, provide much more constraint on the explanation for the inhibitory effects.

Our present experiments also suggest that the first and second phases, though different in character, may start simultaneously with glucose stimulation (64). Thus, measurements of summated first-phase insulin release made over a finite interval could include a contribution by second-phase release. This may explain why the height of the first-phase release (mean early peak) was less affected by the inhibitors than the summated first-phase release. Consideration of this overlapping of the two phases was automatically made in the compartmental computer program, and the computer results closely approximated the biological responses.

The chemical mechanisms of action of diazoxide and DPH are obscure. In time it may be possible to relate these chemical actions to the "sites" of inhibition indicated by model analysis of kinetic secretion patterns.

Diazoxide, among its other actions, affects calcium uptake (77) and cyclic AMP levels (78). This drug also causes blockade at specific steps in glycolysis (79). DPH is known to affect ion flux, possibly by acting on sodium-potassium-dependent ATPase (80).

Finally, as previously suggested (10), other bases for insulin secretion models may be applicable, such as feedback inhibition (1) and differential signal levels (10). Nevertheless, the compartmental model, using a single set of constants derived from a series of glucose dose-response curves (10,17), tested with the superimposed effects of inhibitors, can simulate a variety of intricate changes in secretion induced during experiments of several types of design.

IV. Results Section

D. Insulin Distribution and Metabolism

1. Introduction

Until this point, this thesis has involved discussion of models of the kinetics and the mechanism of insulin secretion. However, to understand the kinetics of the concentration of insulin in the blood *in vivo*, one must combine quantitative models for both the secretion of insulin and the metabolism and distribution of insulin. Thus, a series of *in vivo* experiments were executed and analyzed to evaluate these later phenomena.

Compartment models for insulin metabolism have been presented by several authors (81-83). These models usually consist of 2, 3 or more compartments, one of which corresponds to the blood plasma. A second compartment may correspond to the extracellular space and a third larger compartment occasionally is included which may correspond roughly to total body water. Insulin is thought to distribute among these compartments and may decay at different rates in different compartments. There is a discrepancy between the results in the literature for tracer and non-tracer experiments which we believe was resolved by S. Genuth (84). He showed that iodine labeled insulin is metabolized at approximately 25% of the rate of unlabeled insulin. These metabolic differences combined with the complex kinetics may have delayed the elucidation of the metabolism and distribution of insulin. However, one model gave a reasonable description of insulin metabolism (83) and our results will be described as they relate to that model.

2. Methods

A recently diagnosed 27 year old juvenile type diabetic male weight

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64 Kg. was selected for this study since his endogenous insulin levels were relatively fixed at a level below 9 μU/ml and showed no rise with either spontaneous or induced hyperglycemia. This facilitates evaluation of the distribution and disposal of exogenously administered insulin whose value for half-life measurements and metabolic clearance rate have not yet been agreed upon by workers in this area (81,82,84-88). The subject was given 18 units of insulin on two separate occasions, once by single bolus and once by a steady 4 hour infusion (Fig. 25). Serum insulin was measured at intervals by radioimmunoassay using the method of Grodsky & Forsham (28). A baseline of endogenous insulin was established for the patient before each experiment.

3. Results

Examination of Figure 25 shows that t1/2 of the decay of insulin-plasma concentration for the bolus gradually increases with time. However, if one assumes that in the steady infusion in Figure 25, the steady state insulin concentration is achieved in three half lives, then the calculated t1/2 is smaller than even during the rapid phase at the start of the bolus. Compartment models for insulin clearance and metabolism have been presented by several authors (81-83). In these experimental data we found that linear two and three compartment models with linear destruction rates from all compartments like some of those in the literature (81,82), each gave a least square fit with the error per point within the experimental range (<10% error per point) for either the bolus alone or the steady infusion alone.

However, none of the linear compartment models which we tried gave a good simultaneous fit for the bolus and the steady infusion data together. (All gave >35% error per point). We found that a two compartment model

(Fig. 26a) with a destruction rate of insulin from the blood controlled by a biomolecular reaction which thus obeys Michaelis-Menten kinetics (Vmax= 103μU/min/ml and Km=418μU/ml) did give a good fit (<10% error per point) to all of the data at once (Figure 25). A 2-compartment model with linear destruction rates in which an insulin binding protein was assumed to exist in the blood instead of a biomolecular reaction controlled destruction rate did not fit the data better than did the linear 2 and 3 compartment models. The model in Figure 26a provides a biologically reasonable explanation for the wide range of clearance rates and half times for insulin which now exist in the literature.

Frost et al. (83) have presented a model similar to the one shown in Figure 26a. The Frost m[illegible]rom Figure 26a in that there is both a linear and a bimo[illegible]nsulin from the blood compartment. (Also not shown, [illegible]ariable endogenous insulin secretion in norma[illegible]tics).

When we fi[illegible] in Fig. 26b to our data (with the same ass[illegible]sulin secretion used for the model in Figure 26a)[illegible] to those of Frost for all parameters except K_3, Km and [illegible]ound that neither the data of Frost nor our data were sufficient to define these parameters uniquely. In fact, moving K_3 from the blood compartment to the 2nd compartment still allowed a satisfactory fit (Figure 26c). Table 4 shows that these parameters vary by a ratio of as little as 4 to 1 and as much as 12 to 1 depending on the starting points used for the fits.

Using the steady state equations shown below:

$$I = B \quad (K(B)) \qquad \text{(For our model in Figure 26a)}$$

$$I = B \quad (K_3 + K(B)) \qquad \text{(Model in Figure 26b)}$$

$$I = B \quad \left(K_1 + K(B) \; - \frac{K_1 K_2}{K_2+K_3}\right) \qquad \text{(For our model in Figure 26c).}$$

(Fig. 26a) with a destruction rate of insulin from the blood controlled by a biomolecular reaction which thus obeys Michaelis-Menten kinetics (Vmax= 103μU/min/ml and Km=418μU/ml) did give a good fit (<10% error per point) to all of the data at once (Figure 25). A 2-compartment model with linear destruction rates in which an insulin binding protein was assumed to exist in the blood instead of a biomolecular reaction controlled destruction rate did not fit the data better than did the linear 2 and 3 compartment models. The model in Figure 26a provides a biologically reasonable explanation for the wide range of clearance rates and half times for insulin which now exist in the literature.

Frost et al. (83) have presented a model similar to the one shown in Figure 26a. The Frost model differs from Figure 26a in that there is both a linear and a bimolecular destruction of insulin from the blood compartment. (Also not shown, the Frost model allows for variable endogenous insulin secretion in normals and, if necessary, in diabetics).

When we fitted this version of the model in Fig. 26b to our data (with the same assumption of a fixed basal insulin secretion used for the model in Figure 26a) we got similar values to those of Frost for all parameters except K_3, Km and Vmax. We found that neither the data of Frost nor our data were sufficient to define these parameters uniquely. In fact, moving K_3 from the blood compartment to the 2nd compartment still allowed a satisfactory fit (Figure 26c). Table 4 shows that these parameters vary by a ratio of as little as 4 to 1 and as much as 12 to 1 depending on the starting points used for the fits.

Using the steady state equations shown below:

$$I = B\ (K(B)) \quad \text{(For our model in Figure 26a)}$$

$$I = B\ (K_3 + K(B)) \quad \text{(Model in Figure 26b)}$$

$$I = B\left(K_1 + K(B) - \frac{K_1 K_2}{K_2+K_3}\right) \quad \text{(For our model in Figure 26c).}$$

where I is the steady infusion rate, and B is the steady state insulin concentration of the blood. The metabolic clearance rate (MBC) in ml/Kg/min is then equal to $\frac{I}{B} \cdot \frac{\text{(Blood Vol.)}}{\text{(Bd. Wt. in Kg.)}}$

Each set of values of the parameters obtained by fitting the two models to our data (Table 4) were tested against the data from other workers. In each case we found the values satisfactory for the data as follows: The data of Figures 1 & 3 of Frost et al. (83), the data of Figure 2 of Sönksen et al. (87), and the data of Figures 1,3, and 4 of Sherwin et al. (81).

4. Discussion

It is not clear from this study whether the rate limiting bimolecular reaction is in the transport, sequestering, or enzymatic breakdown of insulin. Isolated liver cell studies in the rat on breakdown of insulin have shown a Km about 120 times higher than our estimate of the Km regulating clearance (89). However, study of the perfused liver has shown saturation at lower concentrations (90). Substrate inhibition (91) and a limitation in transport or sequestering of insulin have been discussed in the literature (92). In one study (83) diabetics were found not to have the saturable bimolecular degradation pathway. Further study will be required to determine why our patient (n=1) still has a saturable pathway. It may be because he is a new diabetic, more than one saturable pathway exists in normals, or the fitted values for model parameters affect this observation. It has been argued (83) that if the liver and kidney cleared all of the insulin of the blood passing thru them, that would not be enough to account for observed clearance rates and so there must be other destruction sites. If insulin is destroyed at its sites of action, then the model shown in Figure 26c seems more conceptually appropriate than the one in Figure 26b or 26a. However,

the model shown in Figure 26a is the simplest of the 3 satisfactory models shown here and the available data does not seem to be sufficient to determine which model is most correct. It is expected that further experiments will provide the constraints necessary to distinguish between these 3 models.

In contrast to another report (88), we find that it is necessary to have the results of a steady infusion study of about 30 minutes duration in addition to the results of a high concentration bolus study to adequately define the insulin metabolic characteristics of an individual patient. For example, one might fit by least squared difference a linear two compartment model with only linear destruction rates to data from a high concentration bolus experiment alone. In that case, we would expect the resultant model to predict too high a steady state blood concentration for an experiment where insulin is infused I.V. at a steady rate in the range of e.g. 10 units or less per hour.

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V. General Discussion

It should be clear to the reader at this point that the mechanism of insulin secretion is not well understood. However, the models presented here are well defined kinetic hypotheses which must be accepted or rejected in the process of elucidating the insulin secretion mechanism. There are a large group of processes which might account for the observed secretion kinetics. The following is an attempt to list the processes involved in association with the appropriate phase of secretion. If the rise of the early phase is considered a separate process, then associated processes might involve, in some unknown order, the rise of cAMP (93), the influx of Ca^{++} (94), the influx of Na^{+} (95), influx of glucose (96), the depolarization of the beta cell (95), the initiation of rapid electrical action potentials (95), the mobilization of internal bound calcium to a free form (97), the inhibition by Na^{+} of mitochondrial Ca^{++} pumping (98), the inhibition of Ca^{++} efflux (99), the formation of glucose metabolic products (100), the interaction of glucose and/or a product and/or Ca^{++} and/or ATP and/or cAMP with any of several receptors (101,100), the suppression of the charge on an insulin granule by Ca^{++} (102), the build up of some gradient across some structure in the beta cell (electrical or concentration gradient) (41), the contraction of microtubules or microfilaments (103), the break down of ATP (40), the binding of vessicles to cell membranes (101), release of insulin from a labile compartment (10), competition of Ca^{++} for sites with polyamines (104), movement of granules closer to the membrane (105), increased granule velocity (105), decreased cytoplasmic vicosity, and other processes. If the fall of the early phase of insulin is a separate process, then it might be associated with depletion of a small labile compartment (10), deactivation

of a rapid secretion signal (41), initiation of mitochondrial Ca^{++} pumping (106), binding of cytosolic Ca^{++} by the cell tissues (97), insulin negative feedback (107), a change in K^{+} permeability or other electrical accomodation (61), a change in Na^{+} permeability (61), local depletion of Ca^{++} by its loss with insulin (108), receptor desensitization (52), dissipation of a gradient across a key cell structure (41), local depletion of cAMP (101), ATP (101), glucose (101), or glucose metabolites (101) and other processes. The late phase of insulin secretion if a separate process, then it might be due to saturation with or decreased pumping of Ca^{++} by mitochondria (106), protein synthesis (3), increased rate of mobilization of stored insulin (10), or some other provisionary process.

No doubt, many of the above processes are involved in secretion but at this time it is difficult to eliminate any of these processes from consideration. Thus, the function of this thesis has been not to solve, but to elucidate the problem. A long series of small steps will be required before the above processes can be organized or eliminated with certainty or even with high probability.

It is clear from this thesis that insulin secretion from the pancreas had the following characteristics: 1) the pancreas secretes in a multiphasic pattern in response to a step in glucose concentration, 2) there is an early phase with sigmoidal dose dependence, 3) the early phase responsiveness can be increased by previous stimulation (hypersensitization or potentiation), 4) there is a late phase with sigmoidal dose dependence, 5) the hypersensitization of the early phase is a process in addition to and maybe independent of provisionary factor P but dependent on many aspects of the history of the pancreas, 6) a ramp in glucose concentration presented slowly enough gives a monophasic secretion response up to a plateau and 7) a rapid

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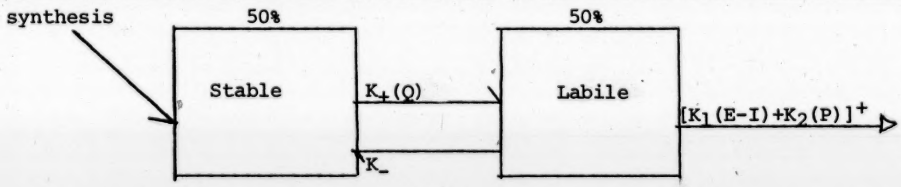
drop in glucose concentration can produce a negative spike in insulin seretion.

The studies above (see Table 1) show that the two-compartment model produces the correct hypersensitivity behavior for only part of the data, the negative spike behavior not at all, and has difficulty refilling the small compartment fast enough in some cases. However, the delta feedback model has no hypersensitization behavior and a simple modification was not satisfactory. Two points should be clear, 1) either model could be elaborated to match all of the data included here (but not necessarily any new experiments, and 2) the characteristics required of a more appropriate model could include properties of both models.

Thus, a reasonable hypothesis for a better model would be a mixture of the two-compartment model and the delta-feedback model (combined model). The following is such a model:



This combined model assumes two compartments, but no small compartment, each compartment containing 50% of the total pancreatic or cell insulin. The early and late phases would be the result of changes in the release constant of the more labile compartment with the general characteristics of the delta-feedback model. Hypersensitivity could occur as most of the contents of the less labile compartment were shifted into the more labile compartment. This process could be controlled by a postulated potentiation parameter Q(G,t) via the exchange rate parameter controlling flow of insulin from the less to more labile compartment.

Due to its larger number of parameters this model would be even less unique than the two-compartment or delta-feedback models. However, given enough experimental data it should be possible to test it further. In the meantime this model represents a conceptual continuum of models in parameter space between the two-compartment and the simple delta-feedback model. For as one reduces the less labile compartment in size the combined model becomes the delta-feedback model. As one reduces the labile compartment in size one must hold the release constant more rigid, move the provisionary term (P) to act on the refilling constant (K_+), to move toward the two-compartment model. With enough data one may be able to determine the unique values of the parameters in the combined model or show why it is unsatisfactory.

The insulin secretion results presented for rate dependence, DPH and diazoxide show that even the above set of combined delta-feedback and two-compartment models are not sufficient structures in their present form. That is, a more appropriate model would be required to exhibit early phase rate dependence, a DPH sensitive refilling, and a third compartment brought into being by diazoxide or some equivalent effects. These results represent 3 additional constraints on insulin secretion models and when the process becomes well enough understood it will no doubt be possible to produce a model which will be consistent with these constraints also. Once again more detailed data will be required. And once again the above models provide a theoretical structure for the experimentalist which can be used to form hypotheses and to ask questions about the process around which to plan experiments.

So then how does the above work relate to the controversies of the day in the study of secretion: How can these models help the experimentalist?

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This work provides a holistic view which indicates the minimum complexity of the insulin secretion system. It helps to tell experimenters what they're looking for (e.g. 2 or more effects of glucose and the list of constraints above) and it can help experimenters know where to look. The above model-represent a group of structures formed using the set of constraints determined from the kinetics of insulin secretion. While the kinetics don't give a final solution, they do give an outline to the puzzle into which other kinds of data can be used to position the pieces. For example, at this time the electrical behavior of the beta cell has been found to exhibit multiphasic kinetics in response to glucose which correlate to insulin secretion kinetics of the pancreas (109). This means so far as secretion and electrical behavior correlate that the techniques for the study of excitable cells can be used to study secretion and that the models presented above can be used to understand the electrical response of the beta cell to glucose and its relationship to secretion. There is controversy among electrophysiologists as to whether the beta cell action potentials are the signal for secretion or the result of secretion by exocytosis. If the action potentials are the signal for secretion this might lend support to a signal limited secretion model where the labile compartment is relatively large and the kinetics of the release constant actually represent the electrical kinetics of the beta cell.

However, if the action potentials are the result of secretion,then the depletion of a small labile compartment could account for the kinetics of the electrical activity. In this way the models may interact with the experiments until secretion is better understood.

In addition to trying to understand the mechanism of insulin secretion, eventually it is hoped that we may understand the relationship of secretion

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kinetics to the action of insulin in normals and diabetics. A satisfactory insulin clearance model would assist in understanding the insulin secretion-action relationship. Thus, we attempted to develop such a model above. Modeling in these two lines of research (secretion and clearance) must be blended together to fully understand the disease state in vivo. As mentioned above this has been tried elsewhere with various simplifying assumptions.

At this time neither secretion nor clearance models have been determined which are unique or sufficient. The models in this thesis are based on in vitro evidence and may or may not be sufficient to describe other stimulators and inhibitors of insulin release. A whole body insulin secretion model would require secretion and clearance modeling of each stimulator and inhibitor of insulin release. Since many of these stimulators and inhibitors interact with each other, a single model including them all would be extremely complex. Thus, one would have a large model with a large number of parameters, using mostly data from in vivo studies which are necessarily imprecise. The result would be a very non-unique model. This reflects science's present true to life experimental view of the system. That is the models are limited by what is known, and thus a complex total body model is not appropriate at this time.

When and if the day comes when effective total body models can be constructed, knowing the parameter values for a given individual will require excessive testing initially and constant updating as the parameters shift with time. Such an effort would probably be worthwhile only if the testing were mechanized or for research purposes. The more feasible route for the near future is to make appropriately limited models for specific medical problems and to use modeling as a research tool.

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Because endocrinology, physiology, and in fact all of *in vivo* biology are necessarily complex, properly applied modeling techniques appear to be an essential tool for the understanding of these problems and the application of this information to medical treatment.

Conclusions

1. Present insulin secretion models must satisfy a complex series of events including fast, transient release, second phase, hypersensitivity and negative response.
2. Specific signal limited secretion models work as well as specific storage limited models.
3. In addition to previously determined constraints (10) an additional hypersensitivity factor Q(G,t))(or equivalent elaboration) is required for sufficient insulin secretion models.
   (a) Q(G,t) has faster kinetics than the provisionary factor, P(G,t).
   (b) Release of catecholamines from nerve endings in the in vitro perfused pancreas does not account for the fast decay of hypersensitivity factor (Q(G,t) at G=0.
4. The in vitro pancreas is sensitive to the rate of change of glucose concentration.
5. The action of diazoxide, including overshoot behavior when the drug is discontinued, can be simulated by a 3* compartment model; whereas a two-compartment model is sufficient to describe the action of DPH.
6. Linear compartment models of in vivo insulin distribution and clearance are not sufficient to simulate both high concentration bolus and steady infusion experiments with a single set of values for the constants.
7. Models with a saturable pathway for the degradation of insulin are sufficient to simulate the clearance from the blood of insulin presented by high concentration bolus and by steady infusion using the single set of values for the constants given in the text above.

*Storage (i.e. a compartment) and release of exciter or signal would be an analogous way for a signal limited model to produce an overshoot.

8. No present insulin distribution and clearance model with a saturable pathway is uniquely defined by the present data.
9. Measurements of insulin levels following both a high concentration insulin bolus and a short steady infusion are necessary to adequately define the insulin metabolic characteristics of an individual patient.
10. A combination of a storage limited model and a signal limited insulin secretion model could simulate all of the experiments presented in this thesis, but such a model would not be unique.

APPENDIX

Assumptions Common to all Models

1. It is valid to use in each model the sigmoidal dose response for the amount of early phase glucose stimulated insulin secretion determined from the data shown in Figure 3.

2. It is valid to use in each model the late phase sigmoid determined from the data in Figure 3.

3. Insulin is stored in one or more compartment in the beta cell of the pancreas.

4. A postulated provisionary factor, P(G,t), exists which controls the late phase secretion rate by changing with first order kinetics to a steady value determined from the data shown in Figure 3.

Notation Common to all Models

Definitions:

t	= time, t = 0 at time of first non-zero glucose concentration (minutes).
$G(t)$	= external glucose concentration (mg%).
$S(G,t)$	= instantaneous insulin secretion rate (μg/min).
$S_1(G,t)$	= one-minute integral insulin secretion rate (μg/min).
$P(G,t)$	= amount of postulated provisionary factor (μg).
K	= 3.3 constant found by the method of least squares fitting to match the sigmoidal relationship between the amount of early phase insulin secretion and the glucose concentration $((mg\%)^K)$.
C	= 1.52×10^7 constant found by the method of least squares fitting to match the sigmoidal relationship between the amount of early phase insulin secretion and the glucose concentration $((mg\%)^K)$.
K_P	= 7.0 constants found by the method of least square fitting to match the sigmoidal relationship between $(P(G,\infty)$ and glucose concentration, $G((mg\%)^{K_P})$.

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C_P = 2.25×10^{15} constants found by the method of least square fitting to match the sigmoidal relationship between $(P(G,\infty)$ and glucose concentration, $G((mg\%)^{K_P})$.

α = $.0337\ min^{-1}$, the growth and decay rate of the postulated provisionary factor $P(G,t)$.

Equations:

$$dP(G,t)/dt = \alpha(P(G,\infty) - P(G,t))$$

$$P(G,\infty) = G^{Kp}/(C_p + G^{Kp})$$

Input-Output:

Input: G(t)

Output: S(G,t)

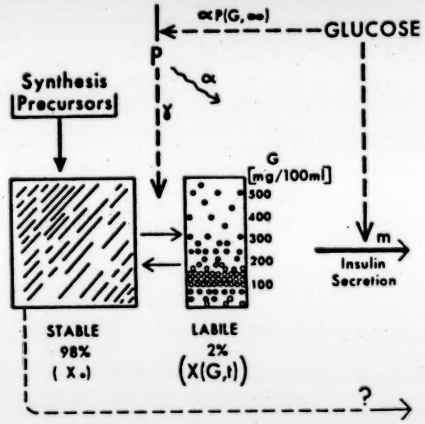
2-Compartment Model

Assumptions

1. Insulin is stored in two compartments.
2. Insulin is stored in a small labile compartment from which insulin is available for immediate release and in a large stable compartment from which the labile compartment is refilled during insulin secretion.
3. The rate of refilling of the labile compartment from the stable compartment is controlled in part by the value of a postulated provisionary factor, $P(G,t)$, which is assumed to follow first order kinetics.
4. Insulin is stored in packets in the two compartments. The packets vary in their threshold sensitivity to glucose for release. $(Z(\theta,0)d\theta$ is the amount of insulin in the packets, for threshold level θ (in units of glucose concentration), between θ and $\theta + d\theta$. The total amount of insulin releasable from the labile compartment at glucose concentration G is then:

$$X(G,0) = \int_0^G Z(\theta,0)d\theta$$

and X(G,0) is the intial integral distribution function which can be determined from the data by plotting to total amount of insulin release during the early phase against the glucose concentration.



2-Compartment Model (Contd.)

Definitions:

$X(G,t)$ = amount of insulin in the labile compartment as a function of glucose concentration G and time t (μg).

$Xs(G,t)$ = amount of insulin in the stable compartment (μg).

$Xmax$ = 1.65 μg, the initial total amount of insulin in the labile compartment.

$K_1(G,t)$ = parameter controlling the rate of exchange of insulin from the stable to the labile compartment (min^{-1}).

$X(G,0)$ = initial amount of insulin available in the labile compartment as a function of glucose concentration (μg). (i.e. initial integral amount of insulin in packets of threshold G or less).

θ = glucose threshold of packets of insulin in the labile compartment. (mg%)

$Z(\theta,0)$ = initial distribution of packets of insulin with glucose threshold for release, θ. (μg/mg%)

K_o = .0002 min^{-1}, the rate constant from the stable to the labile compartment before stimulation with glucose, i.e. when $P(G,t)=0.0$.

$X_s max$ = 82.5 µg, initial total amount of insulin in stable compartment

γ = .5 (min^{-1}) the effectiveness of the postulated provisionary factor, P(G,t).

K_2 = .01 min^{-1}, the rate constant

m = .622 min^{-1}, the release rate constant for insulin secretion from the small compartment.

Equations:

$$dX(G,t)/dt = K_1(G,t)\ X_s(G,t) - (K_2 + m)\ X(G,t)$$

$$K_1(G,t) = K_o + \gamma\, P(G,t)/X_s(G,0)$$

$$X(G,0) = \int_0^G Z(\theta,0)d\theta = Xmax\ G^K/(C + G^K)$$

$$X_s(G,0) = X_s max\ G^K/(C + G^K)$$

$$dX_s(G,t)/dt = K_2\ X(G,t) - K_1(G,t) X_s(G,t)$$

$$S(G,t) = m\ X(G,t)$$

Initial Conditions:

$t = 0$

$K_o X_s(G,t) = K_2 X(G,t)$

$P(G,t) = 0.0$

$X_s(G,t) = X_s(G,0)$

$X(G,t) = X(G,0)$

Delta Signal Model

Assumptions

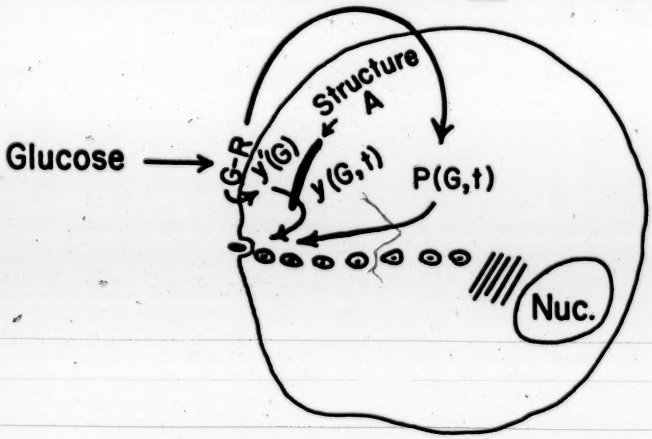
1. Insulin is stored in a single compartment.

2. The difference in amount of some product of glucose across a key cell structure controls the release parameter and thereby determines the rate of early phase insulin secretion. Call it structure A.

3. The difference across structure A approaches equilibration within 5 minutes after a change in external glucose concentration.

4. There is a slow changing provisionary component of the insulin secretion signal which controls the release parameter and which approaches a steady state after 60 minutes of constant external glucose concentration.

5. The transport of insulin to a position in the cell where it is immediately available for release is <u>not</u> a rate limiting process.



G-R = glucose receptor complex

P(G,t) = provisionary factor

Y(G,t) = amount of the glucose product inside of structure A

Y'(G) = amount of the glucose product outside of structure A.

Nuc. = Nucleus

$Y'(G)$ is assumed to reach its value instantaneously and $Y'(G) = Y(G,\infty)$ in magnitude. Thus, the magnitude of the difference across structure A is $Y(G,\infty)-Y(G,t)$.

<u>Definitions</u>:

$Y(G,t)$ = amount of a product of glucose on the inside of some cell structure (μg).

Y_{max} = 1.65 μg, fitting constant chosen before the lease squares fitting of K and C to the sigmoidal relationship between $Y(G,\infty)$ and G.

D_o = .5 min^{-1}, constant determining the rate of increase (or decay) of $Y(G,t)$ inside of some cell structure.

D_1 = .52 min^{-1}, fitting constant relating the difference in $Y(G,t)$ across some cell structure to the instantaneous insulin secretion rate, $S_o(G,t)$.

D_2 = 1.3 $(\mu g/min)^{-1}$, fitting constant relating the product of P(G,t) and the limit of Y(G,t) as t goes to infinity, $Y(G,\infty)$, to the instantaneous secretion rate, S (G,t).

Equations:

$dY(G,t)/dt = D_o(Y(G,\infty)-Y(G,t))$

$Y(G,\infty) = Ymax\ G^K/(C+G^K)$, (note that $Y(G,\infty)$ is proportional to X(G,0) above.)

$S(G,t) = ((D_1(Y(G,\infty)-Y(G,t))+D_2Y(G,\infty)P(G,t))^+$

Initial Conditions:

t = 0

Y(G,t) = 0

P(G,t) = 0

Modified Delta Signal Model:

Same at the delta signal model above except that $S(G,t)=[(D_1(Y(G,\infty)-Y(G,t))+D_2Y(G,\infty)P(G,t)+D_3(Y(G,\infty)-Y(G,t))P(G,t)]^+$

D_3 = 3.5 $(\mu g.min)^{-1}$, fitting constant which adjusts the amount of hypersensitivity behavior of the model so as to match the hypersensitization of the second spike in the experiment shown in Figure 6.

Excitation-Inhibition (Feedback) Model

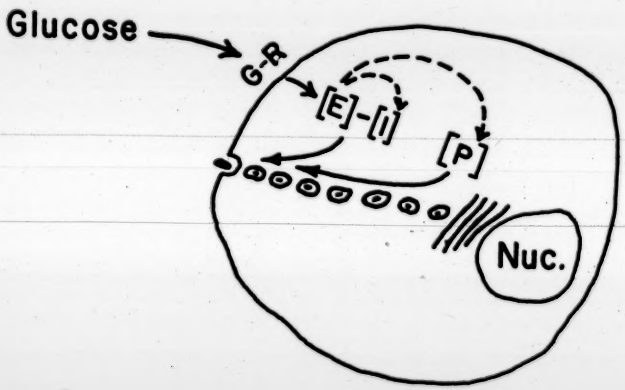
Assumptions

1. Insulin is stored in a single compartment. The difference between the amounts of an exciter, E(G,t), and of an inhibitor, I(G,t), molecule at some key site inside the beta cell controls the release constant and thereby determines the rate of (early phase) insulin secretion. Call it site A.
2. The amount of exciter at site A quickly approaches a new steady state value when the external glucose concentration is changed.

3. Several minutes (5) after a fast increase or decrease in the amount of exciter the slowly changing amount of the inhibitor approaches the amount of the exciter.

4. There is a very slowly changing, internally mobilized, resultant of the exciter to which the inhibitor concentration does not respond which controls the release parameters and which approaches a steady state after 60 minutes of constant external glucose concentration.

5. The transport of insulin to a position in the cell where it is immediately available for release is <u>not</u> a rate limiting process.



G-R = glucose receptor complex
E = exciter
I = inhibitor
P = provisionary factor
Nuc. = nucleus

<u>Definitions</u>:

E(G,t) = amount of exciter at glucose concentration G and time t (µg).

I(G,t) = amount of inhibitor at glucose concentration G and time t (µg).

Emax = 1.65 µg, constant associating the maximum integral amount of insulin in the early phase to the upper limit of the sigmoid relating $E(G,\infty)$ and G.

H_o = 3.0 min^{-1}, constant determing the rate of increase (or decay) of the amount of exciter, E(G,t).

H_1 = .6 min^{-1}, constant determining the rate of increase (or decay) of the amount of inhibitor, I(G,t).

H_2 = .24 $(\mu g/min)^{-1}$, fitting constant relating the product of postulated provisionary factor P(G,t) and E(G,t) to the instantaneous secretion rate S(G,t).

H_3 = 1.0 min^{-1}, fitting constant relating the difference between the exciter E(G,t) and the inhibitor I(G,t) to the instantaneous insulin secretion rate S(G,t).

N = .95, constant determining the minimum secretion rate at the end of the early phase of secretion (no units).

Equations:

$dE(G,t)/dt = H_0(E(G,\infty)-E(G,t))$

$dI(G,t)/dt = H_1(I(G,\infty)-I(G,t))$

$E(G,\infty) = Emax\ G^K/(C+G^K)$, (note that $E(G,\infty)$ is proportional to X(G,0) of the 2-compartment model.)

$I(G,\infty) = N\ E(G,\infty)$

$S(G,t) = (H_3(E(G,t)-I(G,t))+H_2P(G,t)E(G,t))^+$

Initial Conditions:

t = 0

E(G,t) = 0

I(G,t) = 0

P(G,t) = 0

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Legends

FIG. 1. Theoretical scheme for insulin secretion incorporating the concept of a distribution of insulin units or packets which release insulin when their threshold to glucose is reached. Open circles in the small compartment represent the distribution of the threshold-sensitive packets. Broken lines in the large compartment are for artistic balance; the distribution characteristics of this compartment are unknown. Figures 1a and b show the form of the model if new insulin preferentially enters the small (figure a) or the large (figure b) compartments. (From Grodsky (10)).

FIG. 2(a). Theoretical scheme for insulin secretion incorporating the concept that insulin is stored in a single compartment and that the release rate is controlled by the kinetics of a signal. The early phase behavior of the signal is controlled by the difference in the amount of some product of glucose across a key cell structure. The late phase part of the signal is controlled by the value of the slowly changing provisionary factor P. (b) A scheme for insulin secretion similar to that in 2(a) (above) except that the early phase part of the release parameter behavior is controlled by the difference in the amount of exciter and inhibiter at the key sites. The exciter is produced rapidly in presence of glucose while the inhibiter is either stimulated directly by glucose or by feedback through the exciter.

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FIG. 3. Insulin secretion during single-step continuous perfusion with glucose. Solid lines show experimental results. Broken line shows results of computer simulations with the 2-compartment model. Dashed line shows simulation with the delta-feedback model (the modified delta signal model did not vary from the dashed line). The line of alternating dots and dashes shows the results of simulation with the exciter-inhibiter model.

FIG. 4(a). The experimental insulin secretion rate plotted against time. Insulin secretion during staircase stimulations with 50 mg/dl increments of glucose. ▮ is mean ± S.E., n=7. (b) Two-compartment model computer simulation of experiment shown in A. (c) Delta-feedback model simulation of the experiment shown in A. (d) Modified delta-feedback model simulation of the experiment shown in A.

FIG. 5(a). The experimental secretion rate plotted against time. Effect of 4 sequential 100 mg/dl glucose square waves of 5 minutes duration with 5 minute periods of 0 mg/dl glucose between. ▮ is mean ± S.E., n=3. (b) Two-compartment model computer simulation of the experiment shown in A. (c) Delta-feedback model simulation of the experiment shown in A. (d) Modified delta-feedback model simulation of the experiment shown in A.

FIG. 6(a). The experimental insulin secretion rate plotted against time. Effect of two sequential 500 mg/dl glucose stimulations.

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Comparing the summated secretion in minutes 4 through 8 of the first peak to the summated secretion in minutes 41 through 45 of the second peak gives average values of 84.6 µg and 132.8 µg respectively. P is less than .02 using paired statistics. ▮ is mean ± S.E., n=5. (b) Two-compartment model computer simulation of the experiment shown in A. (c) Delta-feedback model simulation of the experiment shown in A. (d) Modified delta-feedback model simulation of the experiment shown in A.

FIG. 7(a). The experimental insulin secretion rate plotted against time. Insulin secretion during a 300 mg/dl glucose step followed by a 5 minute rest at 0 mg/dl followed by 5 minutes at 300 mg/dl. ▮ is mean ± S.E., n=9. (b) Two-compartment model computer simulation of the experiment shown in A. (c) Delta-feedback model simulation of the experiment shown in A. (d) Modified delta-feedback model simulation of the experiment shown in A.

FIG. 8(a). The experimental insulin secretion rate plotted against time. Insulin secretion during two 7 minute 300 mg/dl glucose square waves followed by 10 minutes rest at 0 mg/dl followed by an 11 minute 300 mg/dl glucose square wave. ▮ is the mean ± S.E., n=6. (b) Two-compartment model computer simulation of the experiment shown in A. (c) Delta-feedback model simulation of the experiment shown in A. (d) Modified delta-feedback model simulation of the experiment shown in A.

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FIG. 9(a). The experimental insulin secretion rate plotted against time. Effect of two sequential 300 mg/dl glucose stimulations. Comparing the summated secretion in minutes 4 through 8 of the first peak to the summated secretion in minutes 41 through 45 of the second peak gives average values of 66.5 µg and 106.6 µg respectively. P is less than .1 (N.S.) using paired statistics. ▮ is mean ± S.E., n=4. (b) Two-compartment model computer simulation of the experiment shown in A. (c) Delta-feedback model simulation of the experiment shown in A. (d) Modified delta-feedback model simulation of the experiment shown in A.

FIG.10(a). The experimental insulin secretion rate plotted against time. Effect of two 10 minute sequential 500 mg/dl glucose square waves following 40 minutes at 150 mg/dl glucose and separated by a 10 minute rest at 150 mg/dl glucose. Comparing the summated secretion during the peaks in minutes 41 through 45 to that in minutes 61 through 65 gives average values of 63.0 µg and 101.6 µg respectively. P is less than .01 using paired statistics. ▮ is mean ± S.E., n=5. (b) Two-compartment model computer simulation of the experiment shown in A. (c) Delta-feedback model simulation of the experiment shown in A. (d) Modified delta-feedback model simulation of the experiment shown in A.

FIG.11(a). The experimental insulin secretion rate plotted against time. Effect of two 10 minute sequential 500 mg/dl glucose square waves following 20 minutes at 150 mg/dl glucose and separated

by a 10 minute rest at 150 mg/dl glucose. Comparing the summated secretion during the peaks in minutes 21 through 25 to that in minutes 41 through 45 gives average values of 82.0 and 159.3 respectively with P less than .005 using paired statistics. ▮ is mean ± S.E., n=5. (b) Two-compartment model computer simulation of the experiment shown in A. (c) Delta-feedback model simulation of the experiment shown in A. (d) Modified delta-feedback model simulation of the experiment shown in A.

FIG.12(a). The experimental insulin secretion rate plotted against time. Effect of glucose administration as a slow ramp. Slope of about 5 mg/dl/min. ▮ is mean ± S.E., n=4. (b) Two-compartment model computer simulation of the experiment shown in A. (c) Delta-feedfack model simulation of the experiment shown in A. (d) Modified delta-feedback model simulation of the experiment shown in A.

FIG.13(a). The experimental insulin secretion rate plotted against time. Effect of glucose administration as a rapid ramp. Slope of about 50 mg/dl/min. ▮ is mean ± S.E., n=3. (b) Two-compartment model computer simulation of the experiment shown in A. (c) Delta-feedback model simulation of the experiment shown in A. (d) Modified delta-feedback model simulation of the experiment shown in A.

FIG.14(a). The experimental insulin secretion rate plotted against time. Effect of glucose administered as a slow ramp from 40 to 100 mg/dl followed by a 5 minute rest followed by a square wave of 100 mg/dl glucose. ▮ is mean ± S.E., n=7. (b) Two-compartment

model computer simulation of the experiment shown in A. (c) Delta-feedback model simulation of the experiment shown in A. (d) Modified delta-feedback model simulation of the experiment shown in A.

FIG.15(a). The experimental insulin secretion rate plotted against time. Effect of 20 minutes at 500 mg/dl glucose followed by 20 minutes at 0 mg/dl glucose followed by two 10 minute square waves of 500 mg/dl glucose separated by a 10 minute rest at 0 mg/dl glucose. Summations of insulin secretion in early phase and did not differ significantly. ▮ is mean ± S.E., n=5. (b) Two-compartment model computer simulation of the experiment shown in A. (c) Delta-feedback model simulation of the experiment shown in A. (d) Modified delta-feedback model simulation of the experiment shown in A.

FIG.16(a). The experimental insulin secretion rate plotted against time. Effect of 20 minutes at 500 mg/dl glucose followed by 20 minutes at 30 mg/dl glucose followed by two 10 minute square waves of 500 mg/dl glucose separated by a 10 minute rest of 30 mg/dl glucose. ▮ is mean ± S.E., n=5. (b) Two-compartment model computer simulation of the experiment shown in A. (c) Delta-feedback model simulation of the experiment shown in A. (d) Modified delta-feedback model simulation of the experiment shown in A.

FIG.17(a). The experimental insulin secretion rate plotted against time. Effect of 20 minutes at 500 mg/dl glucose followed by 20 min-

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utes at 0 mg/dl glucose followed by two 10 minute square waves of 500 mg/dl glucose separated by a 10 minute rest at 0 mg/dl glucose. Summations of insulin secretion in early phase and did not differ significantly. ± is mean ± S.E., n=4. The rats were treated with 6-OH-Dopamine 3 days before perfusion of the pancreas. (b) Two-compartment model computer simulation of the experiment shown in A. (c) Delta-feedback model simulation of the experiment shown in A. (d) Modified delta-feedback model simulation of the experiment shown in A.

FIG.18(a). The pattern of glucose perfusion and the plot of insulin secreted by the pancreas as functions of time. A two-step simulation of identical limits and duration was presented first and an eleven-step, ramp-type stimulation was presented second. (b) A plot of the alternate experimental design in which the order of the stimulations was reversed.

FIG. 19. Total insulin secreted when glucose was presented at different maximum rates of change. Data for each bar were derived from the insulin secreted during the shaded periods shown left to right in Fig. 18(a) and (b) and the combined amounts of Fig.18 (a) and (b). Note that in each case more insulin is secreted in response to a two-step change than in response to an eleven-step, ramp-type change. Probability values were calculated using paired Student's t tests.

Table 1. Comparison of the performance of each model in simulation of the various categories of experimental kinetic insulin secretion patterns. + indicates a satisfactory simulation. - indicates an unsatisfactory simulation.

Fig. 20. Effects of inhibitiors on biphasic glucose induced insulin release. In this and subsequent figures DPH=diphenylhydantoin. Shaded bars represent glucose (300 mg/dl).

Fig. 21. Effect of inhibitors on early release of insulin.

Fig. 22. Influence of inhibitors on insulin response to sequential glucose pulses. Shaded bars represent glucose (300 mg/dl).

Fig. 23. Two-compartment model for glucose-induced insulin secretion. Percentages beside symbols in DPH and diazoxide (8) models indicate postulated percentage alteration of control values (fig. 1b) by inhibitors.

Fig. 24. (a)Computerized simulations of biological data from fig. 20, based on 2-compartment model. (b) computerized simulations of biological data from fig. 21, based on 2-compartment model. (c) computerized simulations of biological data from fig. 22, based on 2-compartment model.

Table 2. Effect of prior infusion and dose of drugs on inhibition of early insulin release. Values are means $\pm$ S.E. Insulin, ng/ml.DPH=diphenylhydantoin. SIM=inhibitor and glucose started simultaneously. PRE=inhibitor infused for 10 min prior to

coinfusion with glucose. In all experiments, glucose 300 mg/dl was the stimulus. Σ2-5=mean sum of values in the first 2-5 min after the start of glucose infusion.

Table 3. Insulin response to sequential pulses of glucose (300 mg/dl). Values are insulin, ng/ml ± S.E. NS=not significant (P>.05). Summated=mean sum of values in the first 2-5 min after the start of glucose pulse. See text and Fig. 22 for description of experimental design.

FIG. 25. Insulin blood concentrations from a single recently diagnosed diabetic patient. ⊖=values for an 18 U I.V. bolus given at t=0, and the solid line is the computer fit using the model shown in Figure 26. ▲=values for a steady I.V. infusion of insulin at a rate of .07 U/Kg/4 hr., and the dashed line is the computer fit using the model shown in Figure 25. The patient's weight was 64 Kg.

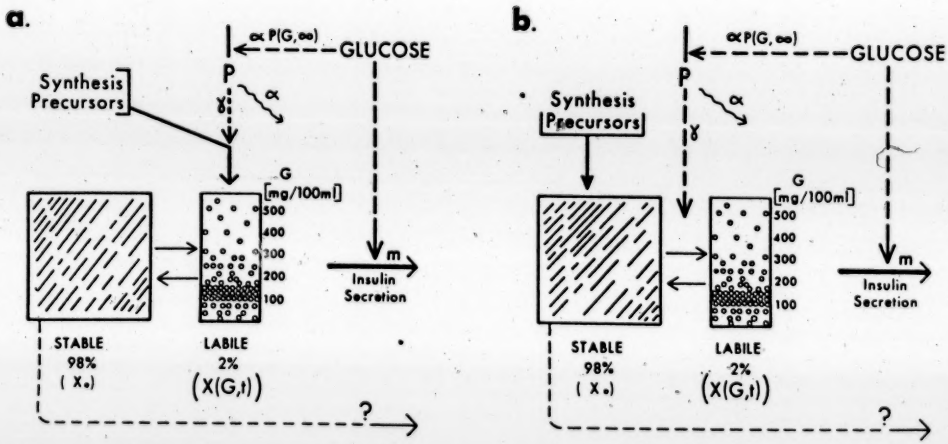
FIG. 26. A model of insulin distribution and degradation in humans. For the steady I.V. insulin infusion experiment the infusion rate, I=.07 U/Kg/4 hr. The patient weighed 64 Kg. For the I.V. bolus experiment I=0, and the initial blood insulin concentration, B(t=0) was assumed to be the 18 U dose divided by the blood volume.

(a) For our patient K_1=.044 min., K_2=.027 min., Vmax=103 μU/min/ml, and Km=418 μU/ml. If passive transport is assumed then the least squares fit gives a plasma associated volume of 7.07 liters, a 2nd compartment of 11.39 liters, and an exchange coefficient of .62. The compartment 2 volume is not incompatible with that of the extra-cellular space.

(b) An insulin metabolism model similar to a model by Frost et al. (9). The values of the parameters depended on the starting values of the fit. See Table 4.

(c) An insulin metabolism model with linear degradation at a site other than the liver or kidney. The values of the parameters depended on the starting values of the fit. See Table 4.

Table 4. Comparison of parameter values for insulin distribution and clearance models fitted to data from one previously undiagnosed diabetic.


a.
∝P(G,∞)
GLUCOSE
Synthesis Precursors
P
γ
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[mg/100ml]
500
400
300
200
100
m
Insulin Secretion
STABLE
98%
(X₀)
LABILE
2%
(X(G,t))
?
b.
∝P(G,∞)
GLUCOSE
Synthesis Precursors
P
γ
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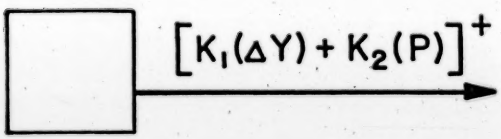
500
400
300
200
100
m
Insulin Secretion
STABLE
98%
(X₀)
LABILE
2%
(X(G,t))
?

FIG. 1

Delta Signal Model



Exciter-Inhibiter Feedback Model

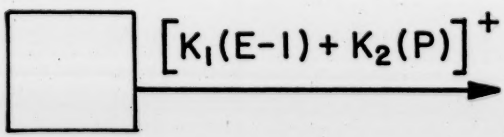


FIG. 2

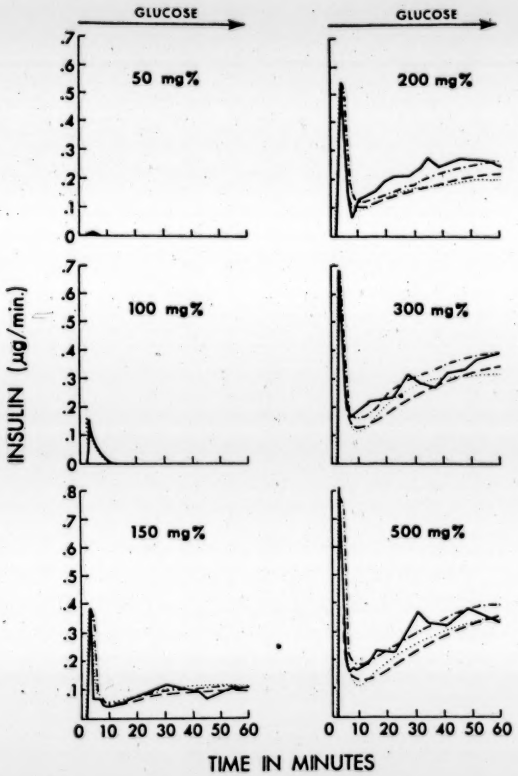

GLUCOSE
GLUCOSE
50 mg%
200 mg%
100 mg%
300 mg%
150 mg%
500 mg%
INSULIN (µg/min.)
0 10 20 30 40 50 60
TIME IN MINUTES

FIG. 3

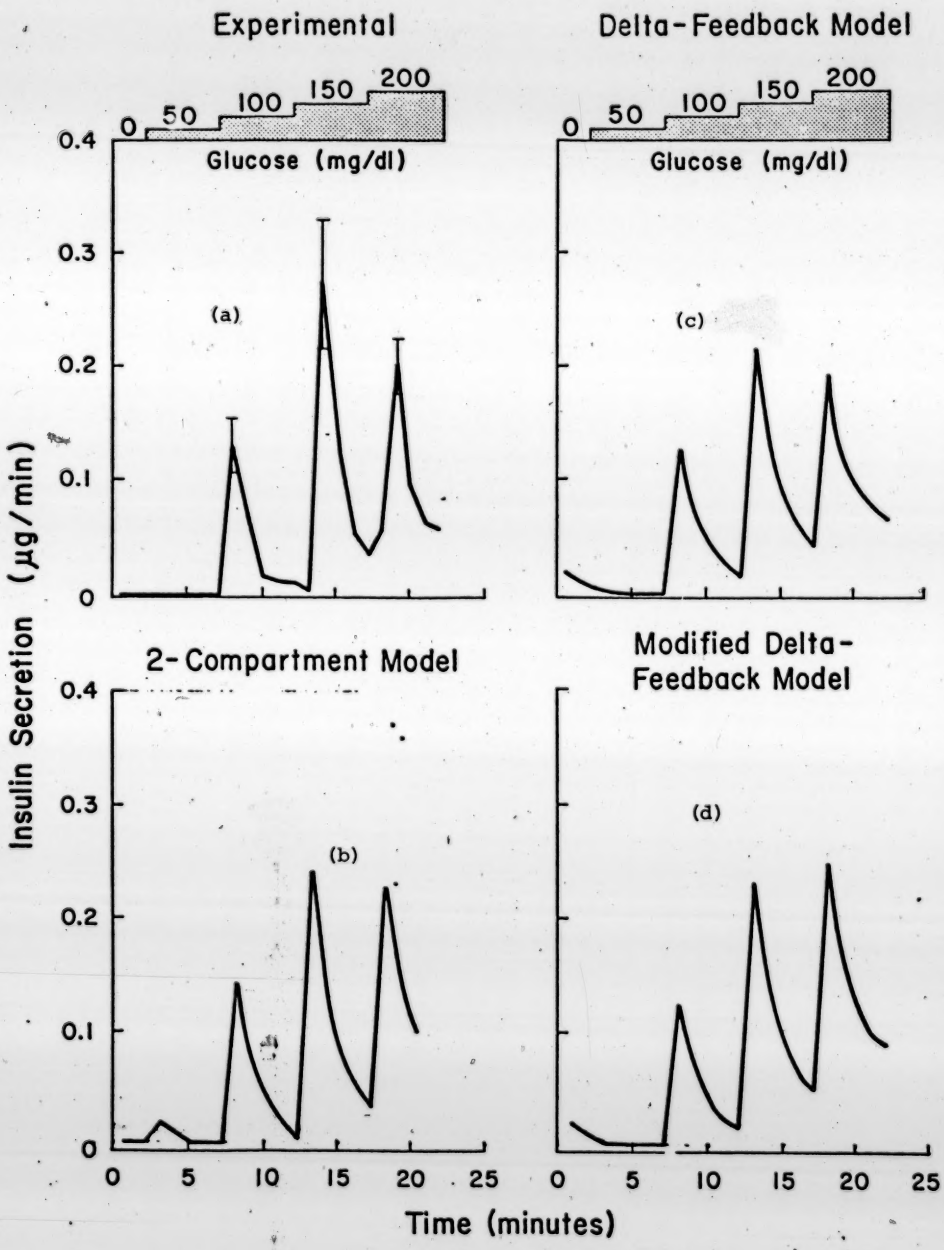

Experimental
Delta-Feedback Model
0 50 100 150 200
Glucose (mg/dl)
(a)
(c)
2-Compartment Model
Modified Delta-Feedback Model
(b)
(d)
Insulin Secretion (μg/min)
0 0.1 0.2 0.3 0.4
0 5 10 15 20 25
Time (minutes)

FIG. 4

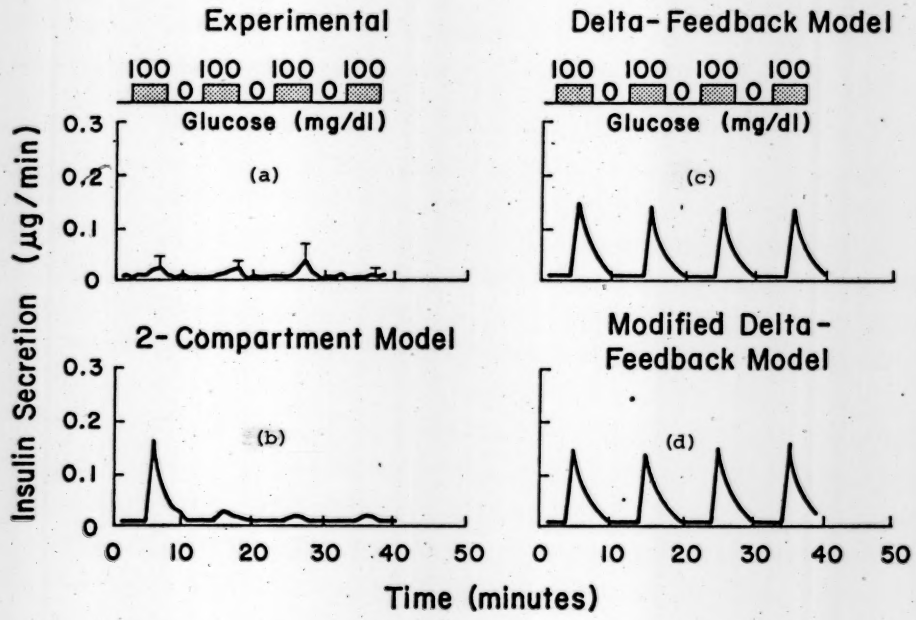

Experimental
Delta-Feedback Model
100 100 100 100
0 0 0 0
Glucose (mg/dl)
(a)
(c)
2-Compartment Model
Modified Delta-Feedback Model
(b)
(d)
Insulin Secretion (μg/min)
0.3
0.2
0.1
0
0 10 20 30 40 50
Time (minutes)

FIG. 5

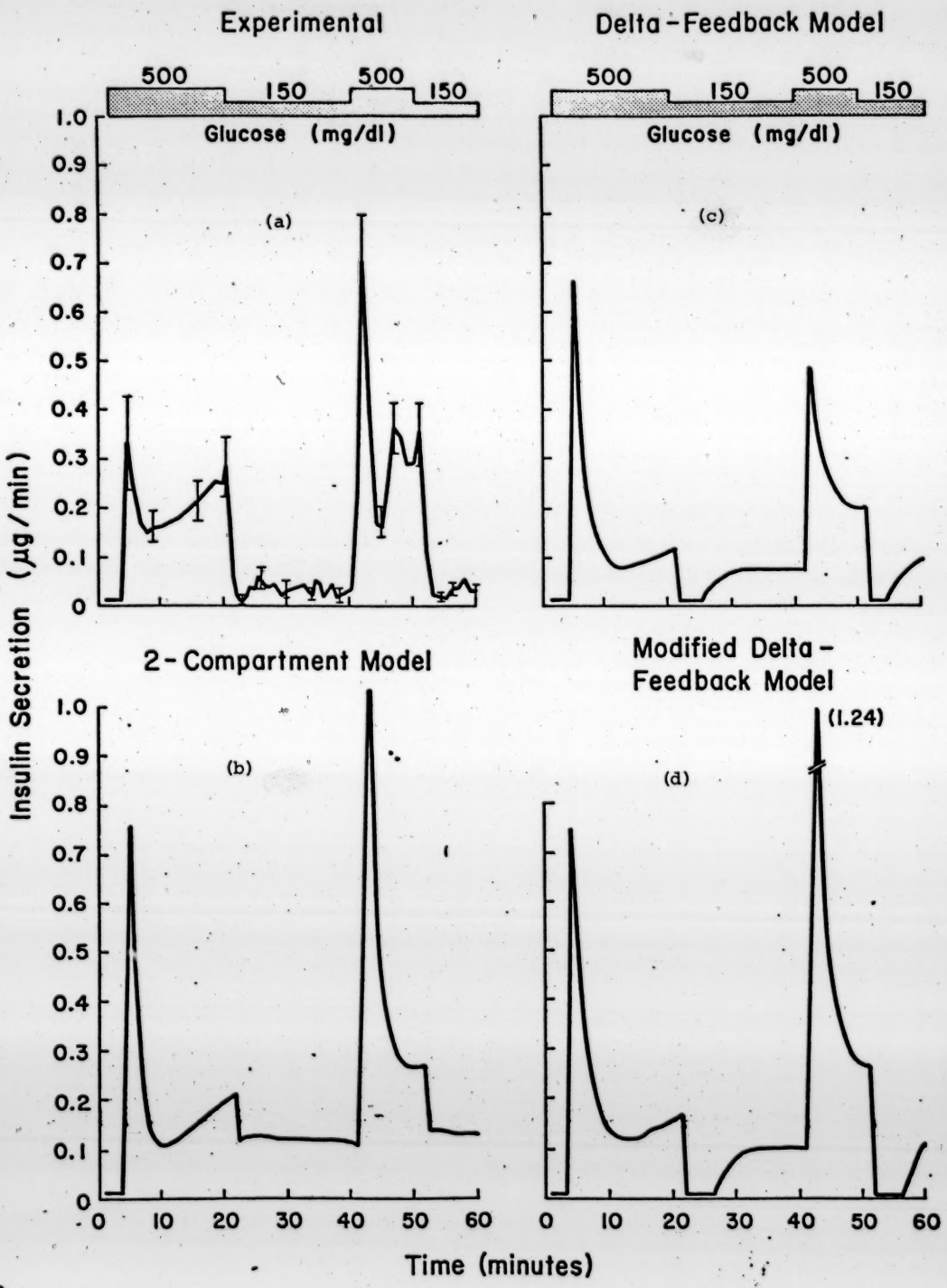

Experimental
Delta-Feedback Model
500
150
500
150
Glucose (mg/dl)
(a)
(c)
2-Compartment Model
Modified Delta-Feedback Model
(b)
(d)
(1.24)
Insulin Secretion (μg/min)
Time (minutes)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
10
20
30
40
50
60

FIG. 6

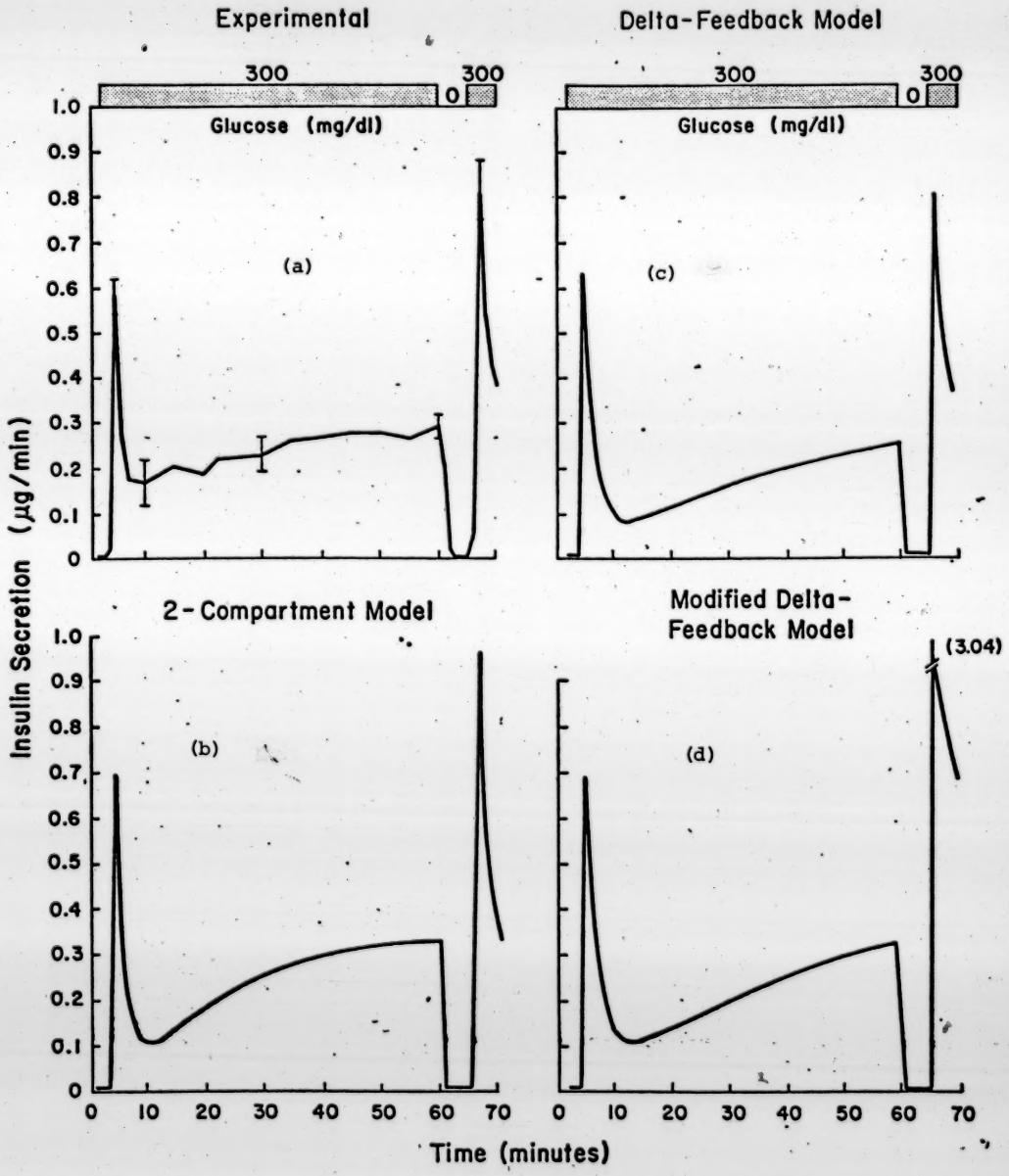

Experimental
Delta-Feedback Model
300
0
300
Glucose (mg/dl)
(a)
(c)
2-Compartment Model
Modified Delta-Feedback Model
(b)
(d)
(3.04)
Insulin Secretion (μg/min)
Time (minutes)

FIG. 7

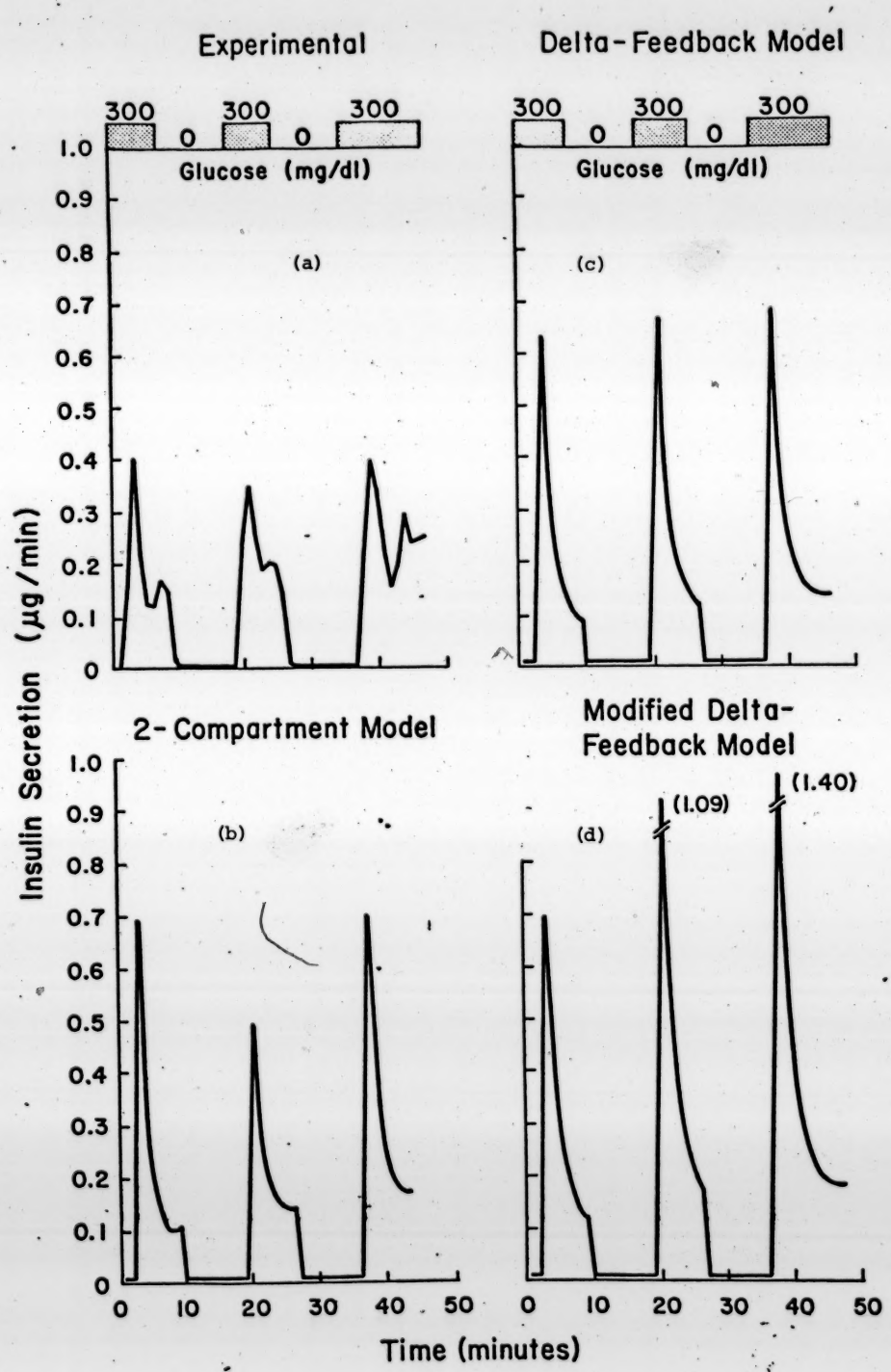

Experimental
Delta-Feedback Model
300
0
300
0
300
Glucose (mg/dl)
(a)
(c)
2-Compartment Model
Modified Delta-Feedback Model
(b)
(d)
(1.09)
(1.40)
Insulin Secretion (μg/min)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
Time (minutes)

FIG. 8

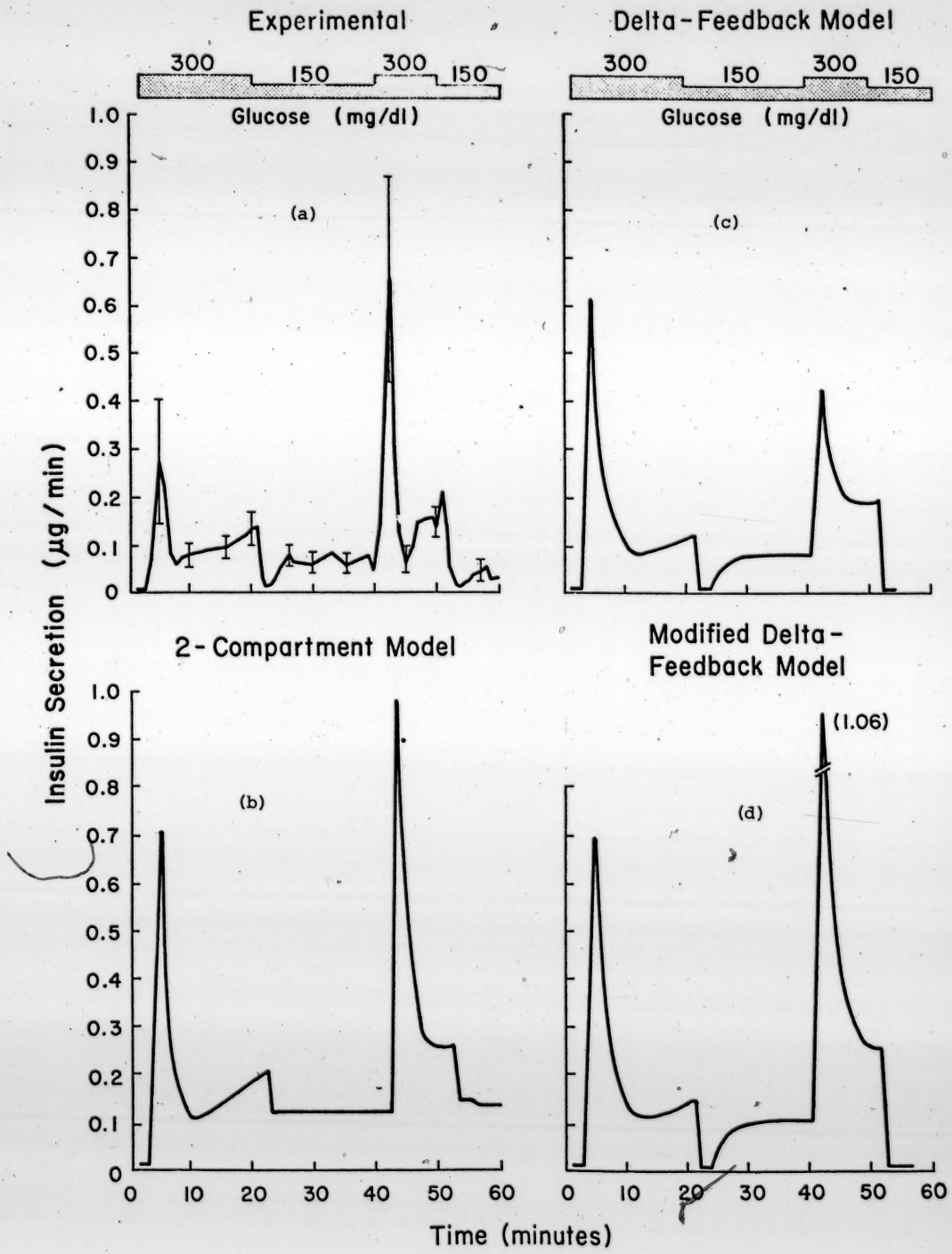

Experimental
Delta-Feedback Model
300
150
300
150
Glucose (mg/dl)
(a)
(c)
2-Compartment Model
Modified Delta-Feedback Model
(b)
(d)
(1.06)
Insulin Secretion (μg/min)
Time (minutes)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
10
20
30
40
50
60

FIG. 9

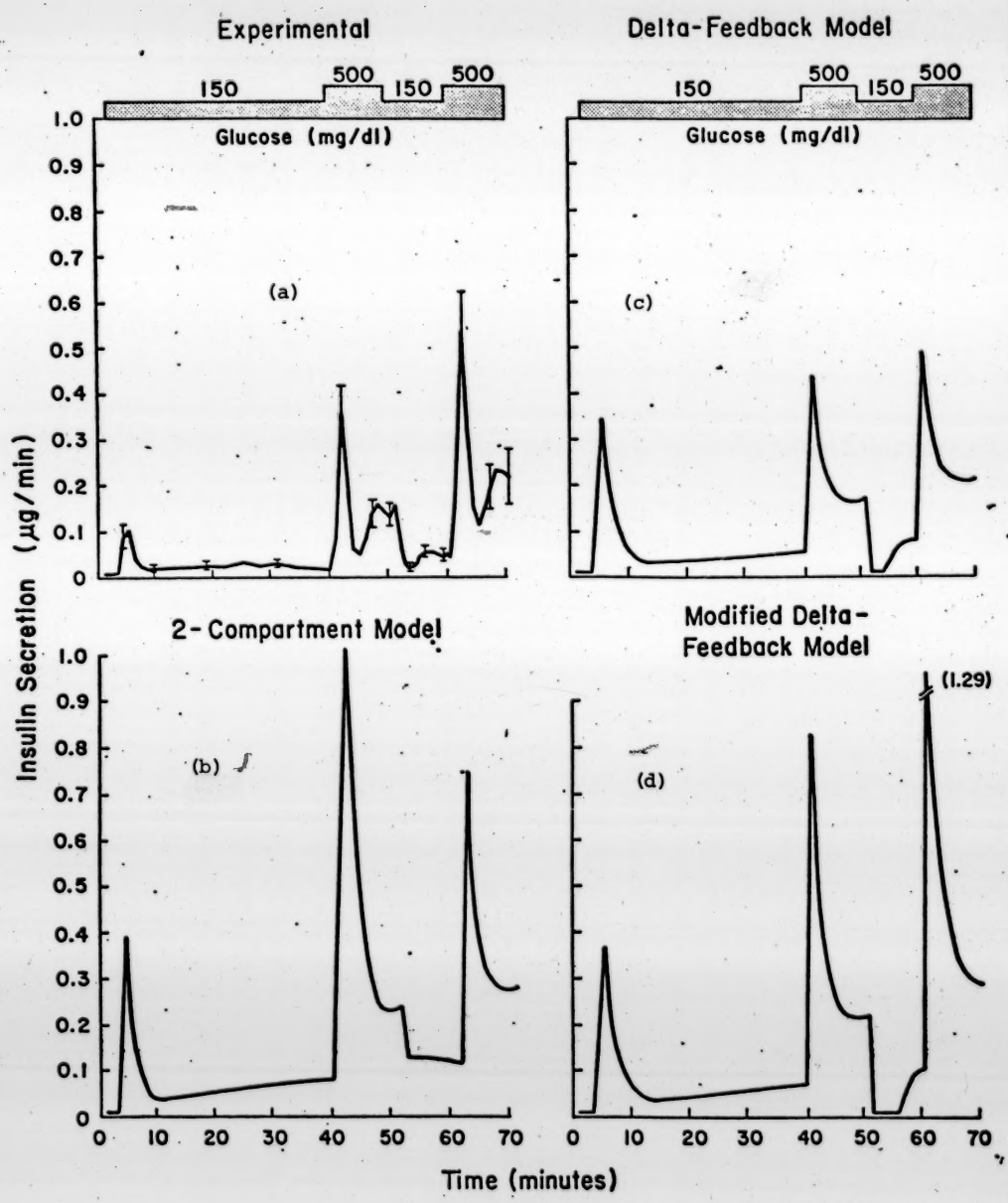

Experimental
Delta-Feedback Model
150
500
150
500
Glucose (mg/dl)
(a)
(c)
2-Compartment Model
Modified Delta-Feedback Model
(b)
(d)
(1.29)
Insulin Secretion (μg/min)
Time (minutes)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
10
20
30
40
50
60
70

FIG. 10

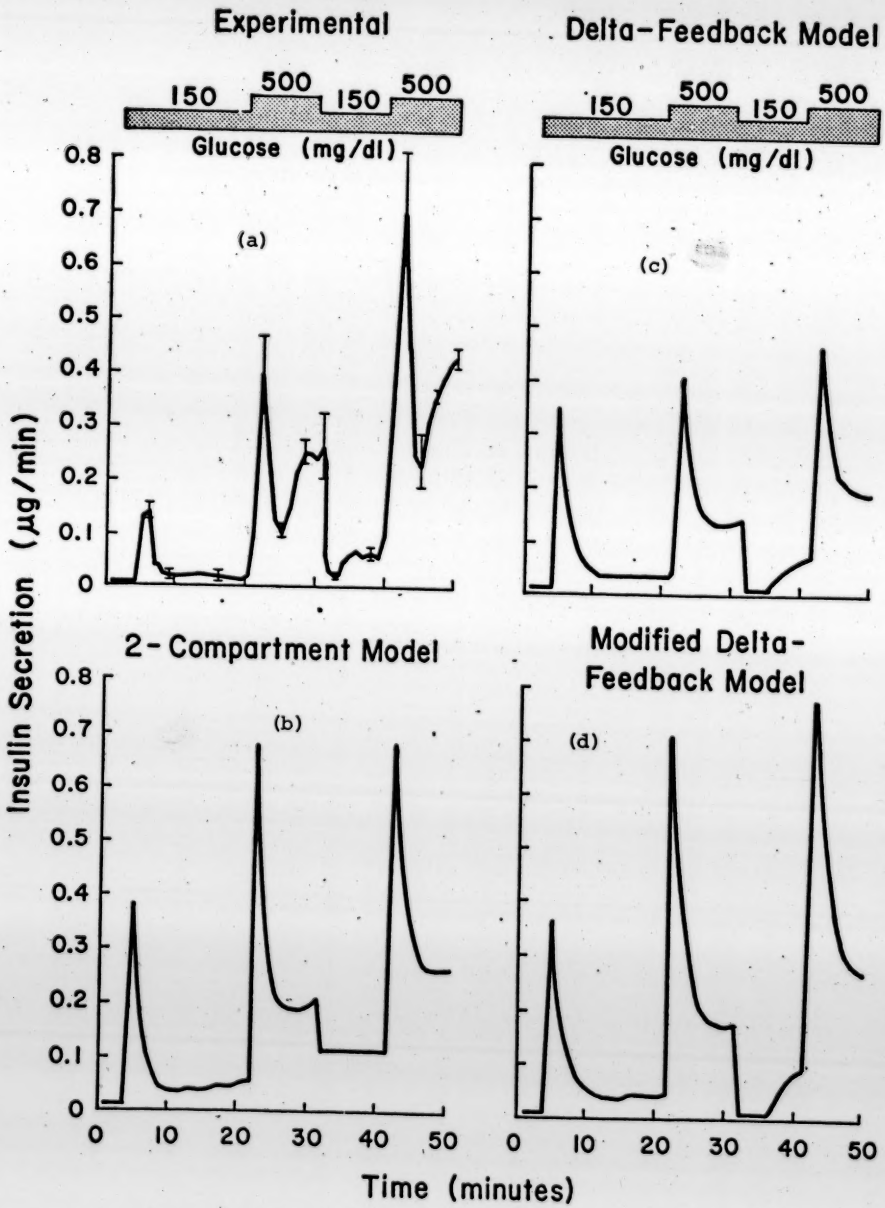

Experimental
Delta-Feedback Model
150
500
150
500
Glucose (mg/dl)
(a)
(c)
2-Compartment Model
Modified Delta-Feedback Model
(b)
(d)
Insulin Secretion (μg/min)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0
10
20
30
40
50
Time (minutes)

FIG. 11

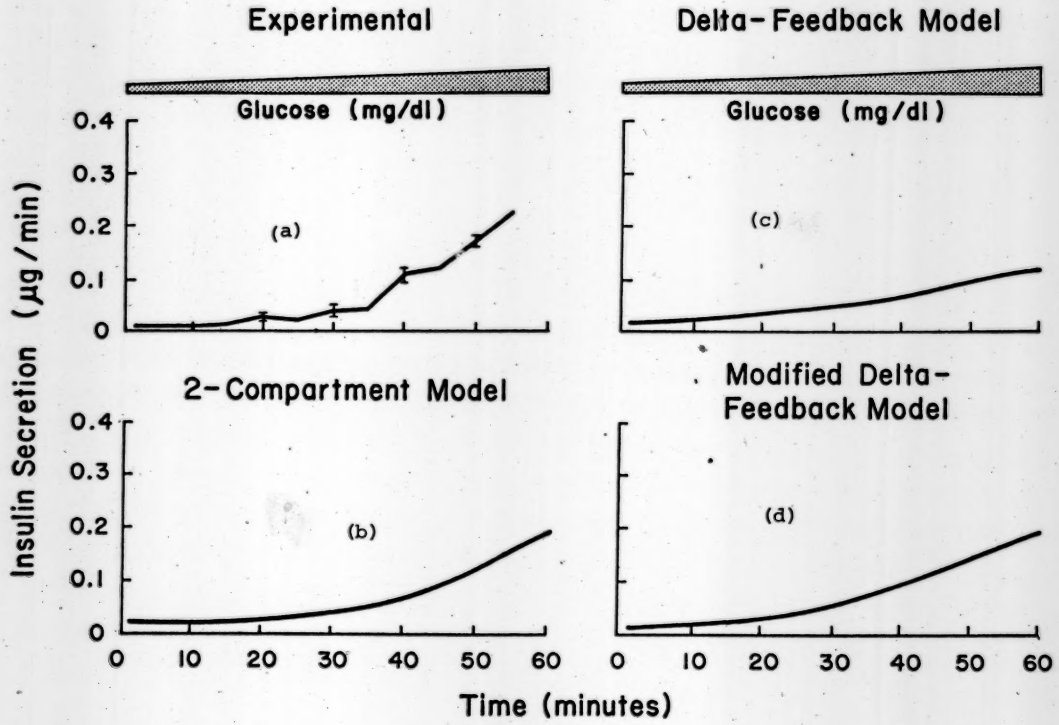

Experimental
Delta-Feedback Model
Glucose (mg/dl)
Glucose (mg/dl)
(a)
(c)
2-Compartment Model
Modified Delta-Feedback Model
(b)
(d)
Insulin Secretion (μg/min)
0.4
0.3
0.2
0.1
0
0 10 20 30 40 50 60
Time (minutes)

FIG. 12

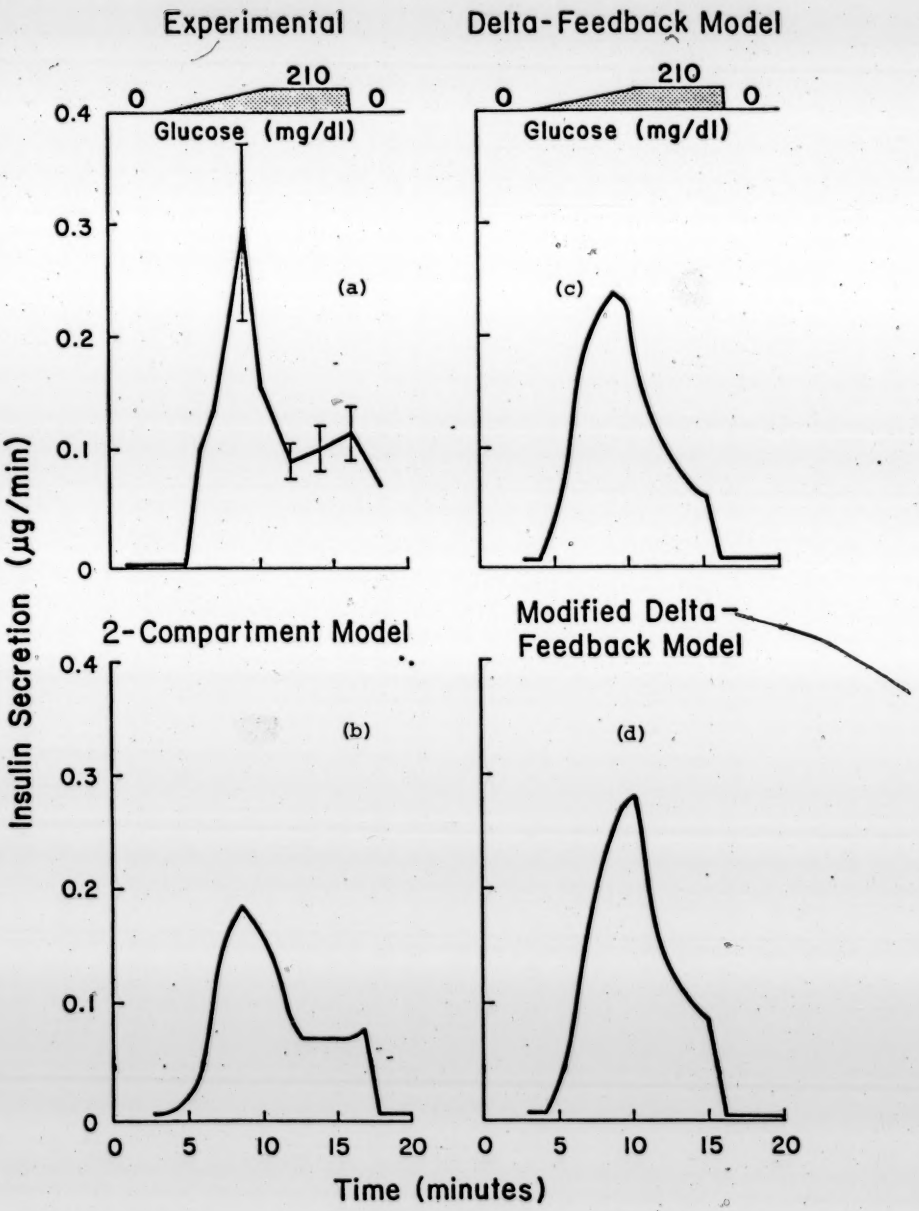

Experimental
Delta-Feedback Model
210
0
0
Glucose (mg/dl)
(a)
(c)
2-Compartment Model
Modified Delta-Feedback Model
(b)
(d)
Insulin Secretion (μg/min)
0.4
0.3
0.2
0.1
0
0
5
10
15
20
Time (minutes)

FIG. 13

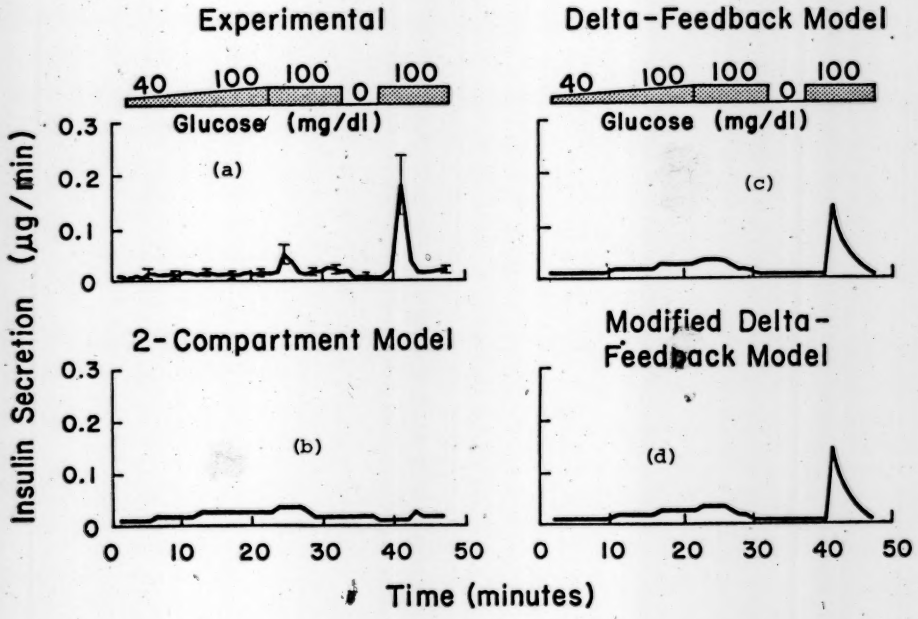

Experimental
Delta-Feedback Model
40 100 100 0 100
Glucose (mg/dl)
(a)
(c)
2-Compartment Model
Modified Delta-Feedback Model
(b)
(d)
Insulin Secretion (μg/min)
0.3
0.2
0.1
0
0 10 20 30 40 50
Time (minutes)

FIG. 14

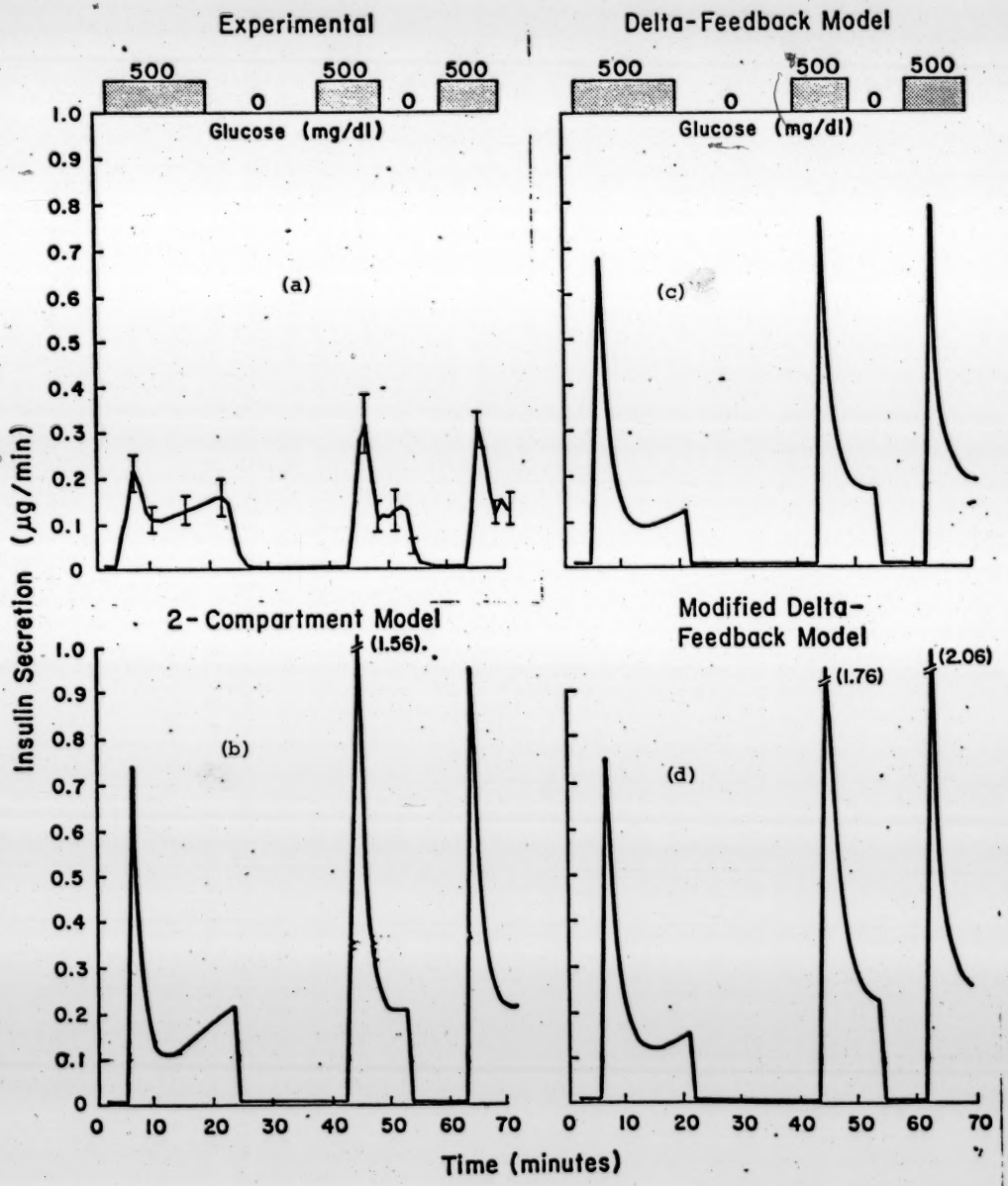

Experimental
Delta-Feedback Model
500
0
500
0
500
Glucose (mg/dl)
(a)
(c)
Insulin Secretion (μg/min)
2-Compartment Model
Modified Delta-Feedback Model
(1.56)
(1.76)
(2.06)
(b)
(d)
Time (minutes)

FIG. 15

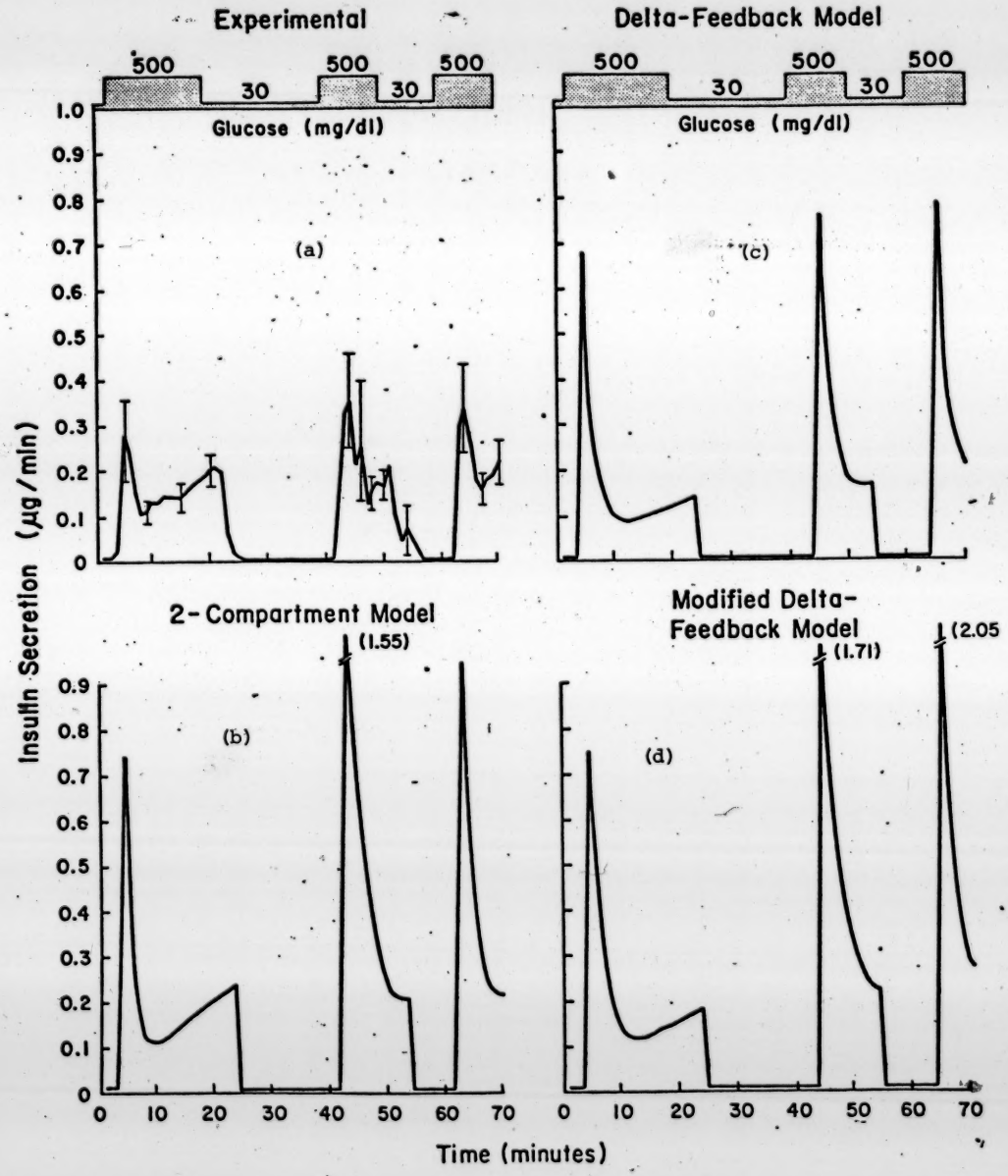

Experimental
Delta-Feedback Model
500
30
500
30
500
Glucose (mg/dl)
(a)
(c)
2-Compartment Model
Modified Delta-Feedback Model
(1.55)
(1.71)
(2.05
(b)
(d)
Insulin Secretion (μg/min)
Time (minutes)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
10
20
30
40
50
60
70

FIG. 16

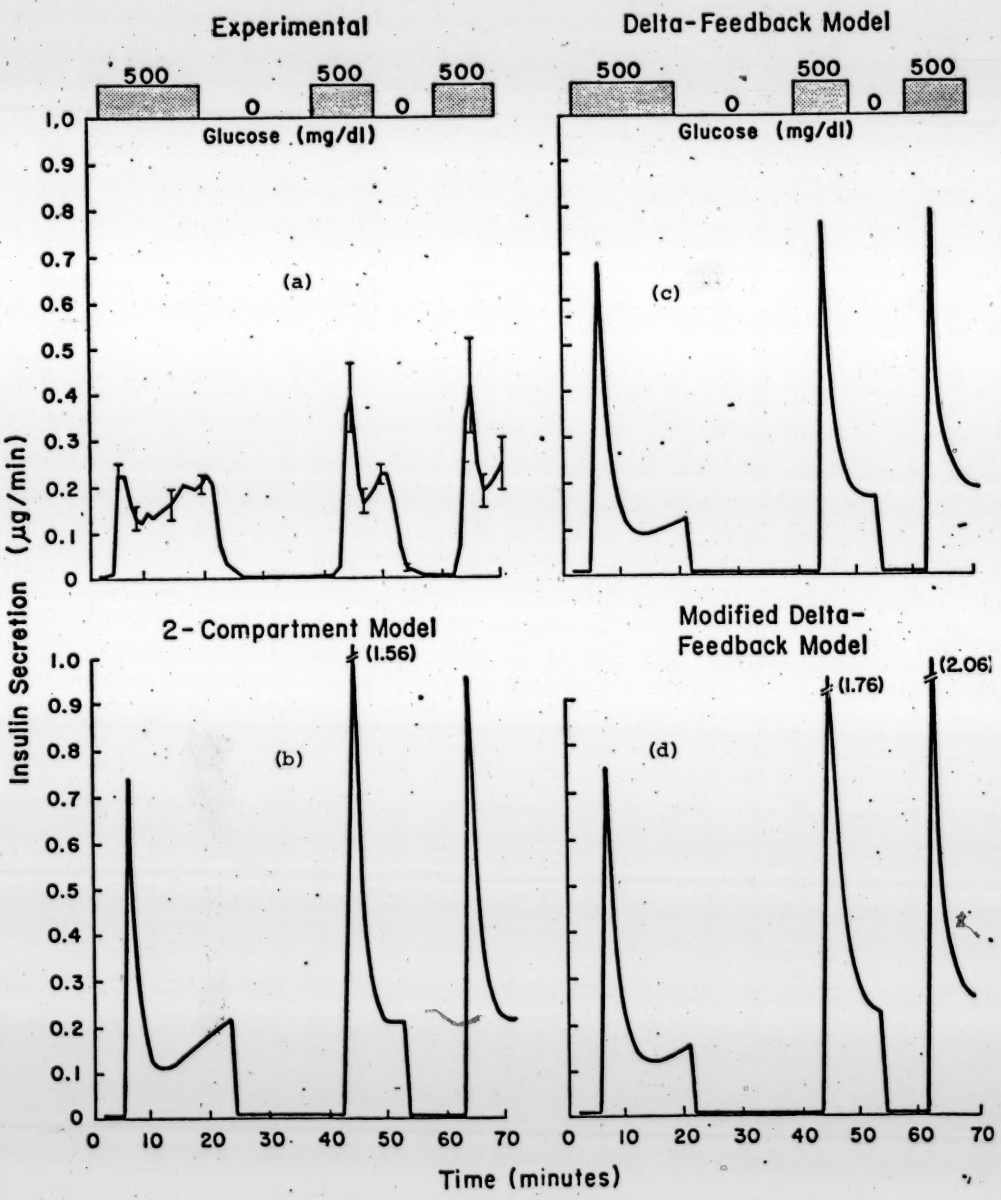

Experimental
Delta-Feedback Model
500
0
500
0
500
Glucose (mg/dl)
(a)
(c)
Insulin Secretion (μg/min)
2-Compartment Model
Modified Delta-Feedback Model
(1.56)
(1.76)
(2.06)
(b)
(d)
Time (minutes)

FIG. 17

Table 1

Type of Behavior	2-Compartment	Delta-Feedback	Modified Delta-Feedback
Early Phase Control			
Dose Response (Fig. 3 & 4)	+ (fitted)	+ (fitted)	+ (fitted)
Late Phase Control			
Dose Response (Fig. 3)	+ (fitted)	+ (fitted)	+ (fitted)
Rest Restimulation (Refilling)			
Fig. 7 (60 -5- 5 min) (300-0-300mg%)	+	+	- (Major Error)
Fig. 5 (Low Concentration Pulses)	-	+	+
Fig. 8 (High Concentration Pulses)	+	+	- (Major Error)
Fig. 6 & 9	+	-	+ (fitted)
Fig. 10 & 11	-	-	+
Fig. 14	-	+	+
Ramps			
Fast (Fig. 12)	+	+	+
Slow (Fig. 13)	+	+	+
Negative Spike			
Fig. 6,9,10, & 11	-	+	+

UMI

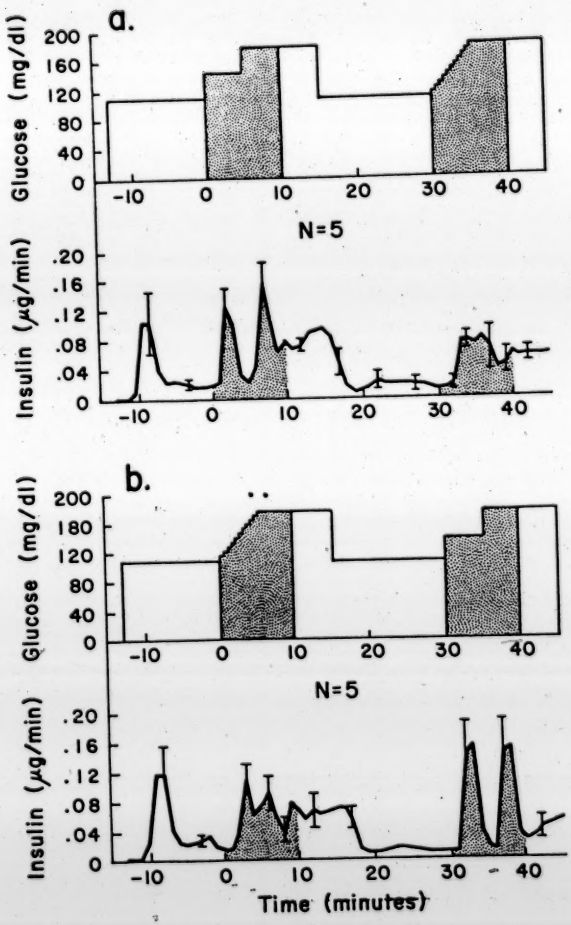

a.
Glucose (mg/dl)
200
160
120
80
40
0
-10
0
10
20
30
40
N=5
Insulin (μg/min)
.20
.16
.12
.08
.04
0
b.
Glucose (mg/dl)
200
180
120
80
40
0
N=5
Insulin (μg/min)
.20
.16
.12
.08
.04
0
Time (minutes)

FIG. 18

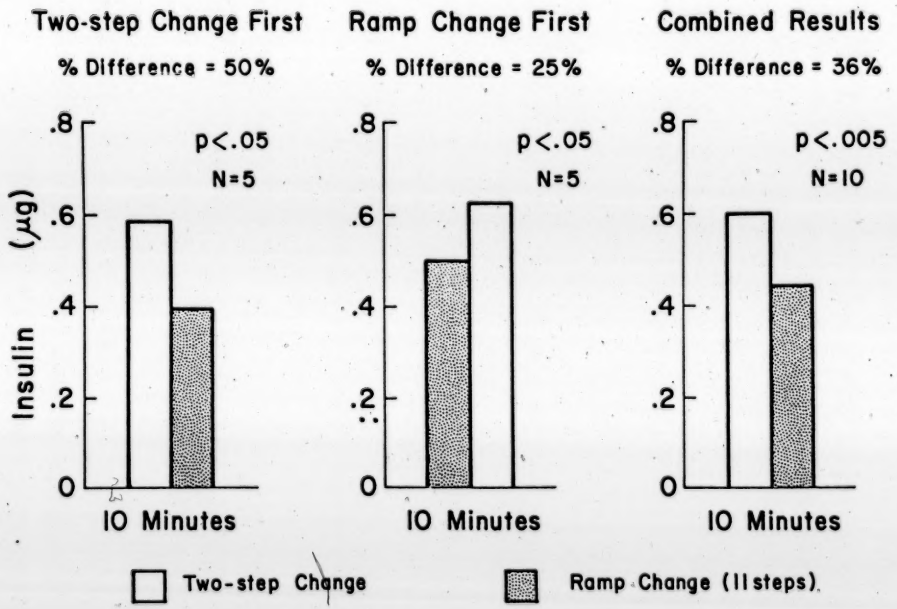

Two-step Change First
% Difference = 50%
p<.05
N=5
Ramp Change First
% Difference = 25%
p<.05
N=5
Combined Results
% Difference = 36%
p<.005
N=10
Insulin (μg)
.8
.6
.4
.2
0
10 Minutes
Two-step Change
Ramp Change (11 steps)

FIG. 19

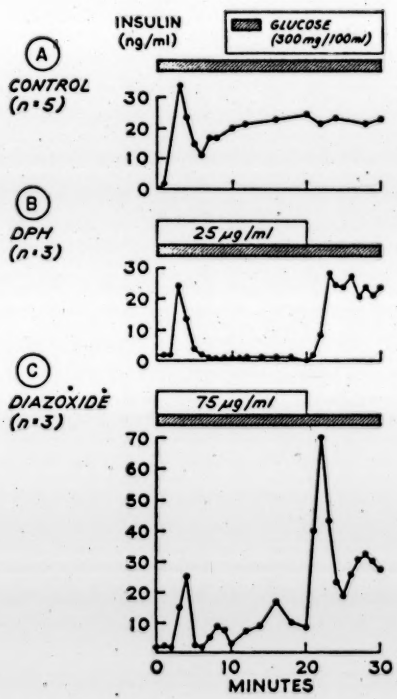

INSULIN
(ng/ml)
GLUCOSE
(300mg/100ml)
A
CONTROL
(n=5)
30
20
10
0
B
DPH
(n=3)
25 µg/ml
30
20
10
0
C
DIAZOXIDE
(n=3)
75 µg/ml
70
60
50
40
30
20
10
0
10
20
30
MINUTES

FIG. 20

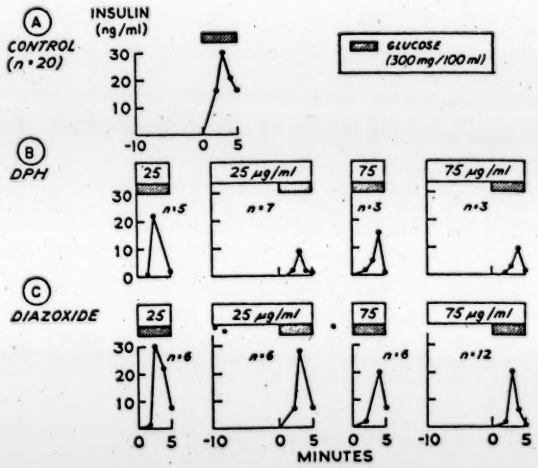

A
CONTROL
(n = 20)
INSULIN
(ng/ml)
30
20
10
-10
0
5
GLUCOSE
(300 mg/100 ml)
B
DPH
25
25 µg/ml
75
75 µg/ml
n=5
n=7
n=3
n=3
C
DIAZOXIDE
n=6
n=6
n=6
n=12
MINUTES

FIG. 21

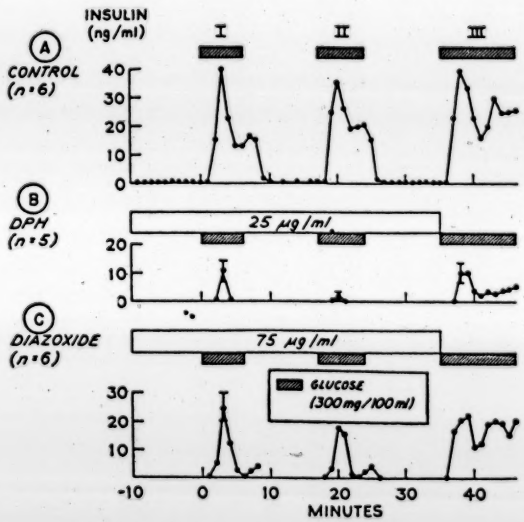

INSULIN
(ng/ml)
I
II
III
A
CONTROL
(n=6)
40
30
20
10
0
B
DPH
(n=5)
25 µg/ml
20
10
0
C
DIAZOXIDE
(n=6)
75 µg/ml
GLUCOSE
(300mg/100ml)
30
20
10
-10
0
10
20
30
40
MINUTES

FIG. 22

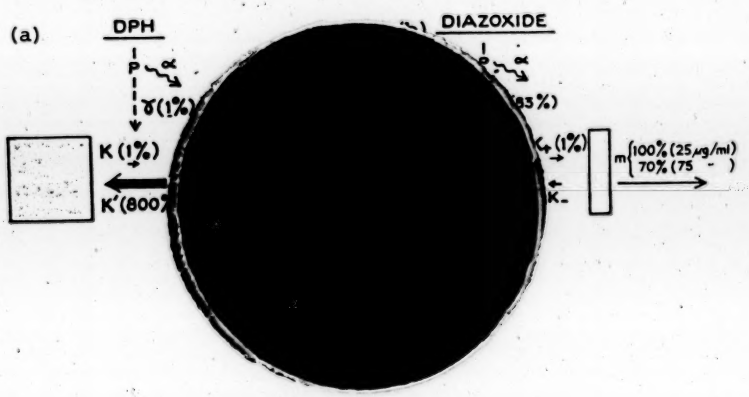


FIG. 23

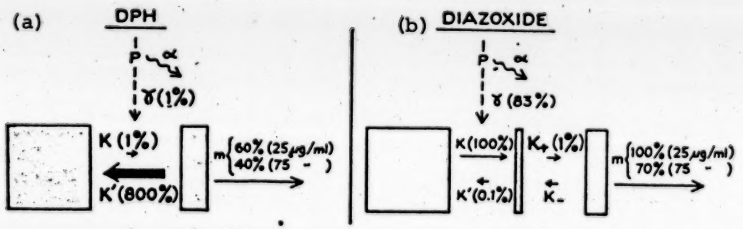

(a)
DPH
P
α
γ(1%)
K (1%)
K'(800%)
m
60% (25 μg/ml)
40% (75 -)
(b)
DIAZOXIDE
P
α
γ (83%)
K(100%)
K'(0.1%)
K+ (1%)
K-
m
100% (25 μg/ml)
70% (75 -)

FIG. 23

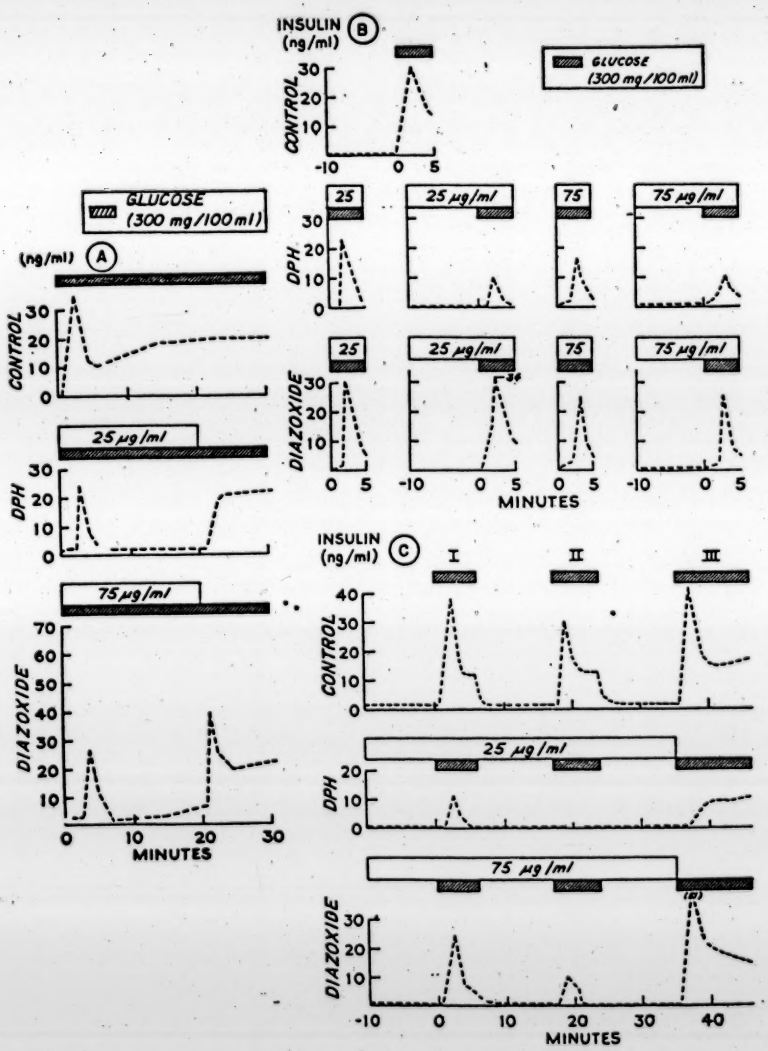

INSULIN (ng/ml)
B
GLUCOSE (300 mg/100 ml)
CONTROL
30
20
10
-10
0
5
GLUCOSE (300 mg/100 ml)
(ng/ml)
A
CONTROL
30
20
10
0
25 µg/ml
DPH
75 µg/ml
DIAZOXIDE
70
60
50
40
30
20
10
0
10
20
30
MINUTES
25
25 µg/ml
75
75 µg/ml
DPH
DIAZOXIDE
-10
0
5
MINUTES
INSULIN (ng/ml)
C
I
II
III
CONTROL
40
30
20
10
0
25 µg/ml
DPH
75 µg/ml
DIAZOXIDE
-10
0
10
20
30
40
MINUTES

FIG. 24

Table 2

Drug	Dose µg/ml	Conditions	n	Summated (Σ2-5)	P vs. Control
		Control	20	83 ± 4	
DPH	25	SIM	5	33 ± 4	<.001
		PRE	7	11.8 ± 2	<.001
	75	SIM	3	19 ± 7	<.001
		PRE	3	13 ± 2	<.001
Diazoxide	25	SIM	6	57 ± 7	<.01
		PRE	6	52 ± 11	<.02
	75	SIM	6	39 ± 7	<.001
		PRE	12	38 ± 4	<.001

Table 3

		I	II	III	P(Paired Analysis) I vs. II	I vs. III	II vs. III
Control (n=6)	Summated	90±3	105±18	111±16	NS	NS	NS
DPH, 25 µg/ml (n=5)	Summated	12±3	3±1	21±7	.03	NS	<.03
Diazoxide, 75 µg/ml (n=6)	Summated	41±4	36±4	65±12	NS	NS	<.03

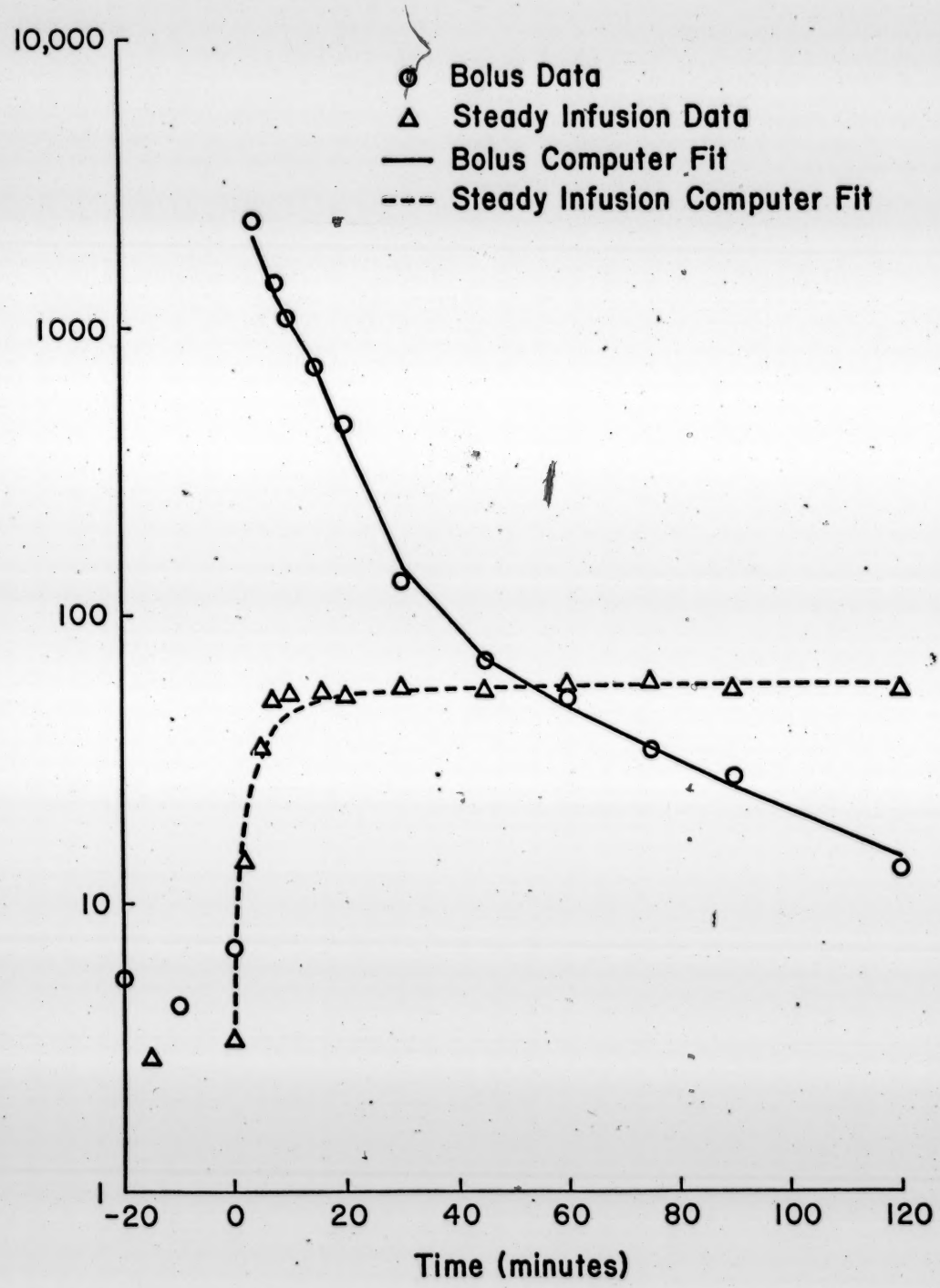
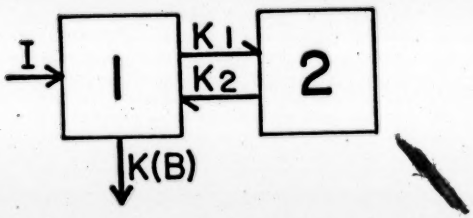

10,000
1000
100
10
1
Bolus Data
Steady Infusion Data
Bolus Computer Fit
Steady Infusion Computer Fit
-20
0
20
40
60
80
100
120
Time (minutes)

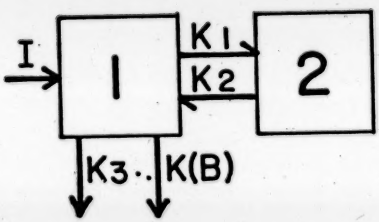
FIG. 25

$$K(B) = \frac{Vmax}{Km + B}$$

a.



b.



c.

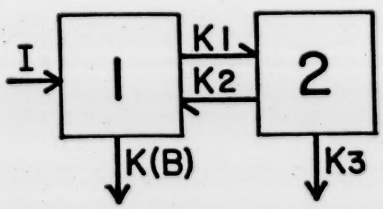


FIG. 26

TABLE 4

COMPARISON OF MODEL PARAMETER VALUES

	Min-1 K_1	Min-1 K_2	Min-1 K_3	μU/ml Km	μU/min/ml Vmax	Liters Blood Related Vol.	LSD	Vmax / Km
Model in Fig. 26a	.0435	.0269	0.0	417.78	103.45	7.071	.0964	.245
Frost et al. original values (for normals)	.120	.058	.109	44.3	16.15	4.77	N.A.	.365
Frost et al.original values (for diabetics)	.0506	.0619	.127	- - -	- - -	9.363	N.A.	
Model in Fig. 26b (using Frost starting values, for normals)	.052	.0248	.0639	61.1	22.9	5.50	.1103	.377
Model in Fig. 26b (using 26a start 26a starting values)	.043	.029	.012	371	88.7	6.82	.0990	.239
Model in 26b (using intermediate starting point)	.0456	.0246	.0373	183.32	46.8	6.33	.1007	.255
Model in 26c (using 26a starting values)	.066	.021	.008	339	87.7	6.24	.0953	.259
Model in 26c (using Frost starting values, for normals)	.0892	.0127	.0122	150.6	38.6	6.33	.0975	.256